WAGNER SOUSA ALVES

CONSUMO, DIGESTIBILIDADE APARENTE E ESTABILIDADE AERÓBIA DE SILAGEM DE SORGO INOCULADA COM DIFERENTES CEPAS DE *Lactobacillus buchneri*

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Zootecnia, para obtenção do título de *Magister Scientiae*.

Orientador: Odilon Gomes Pereira

Coorientadora: Karina Guimarães Ribeiro

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RESUMO

ALVES, Wagner Sousa, M.Sc.. Universidade Federal de Viçosa, julho de 2019. **Consumo, digestibilidade aparente e estabilidade aeróbia de silagem de sorgo inoculada com diferentes cepas de *Lactobacillus buchneri***. Orientador: Odilon Gomes Pereira. Coorientadora: Karina Guimarães Ribeiro.

Foram realizados dois experimentos com objetivo de avaliar o efeito de diferentes cepas de *Lactobacillus buchneri* sobre a estabilidade aeróbia de silagem de sorgo em diferentes períodos de fermentação (Experimento 1) e o consumo, digestibilidade aparente, balanço de nitrogênio e fermentação ruminal em carneiros alimentados com silagens de sorgo inoculadas com cepas de *L. buchneri* (Experimento 2). No experimento 1, o sorgo foi ensilado em dois anos consecutivos após a aplicação dos seguintes inoculantes: Controle; Lalsil AS; e três cepas de *L. buchneri*: LB 45.22, LB 50.4 e LB 90.14. Os mini silos foram abertos após 20, 40 e 60 dias de fermentação. O teor de ácido lático foi menor aos 20 d de fermentação nas silagens inoculadas com as cepas LB 45.22 e LB 90.14. Essas silagens também apresentaram os maiores teores de ácido acético e a menor população de leveduras aos 20 d de fermentação. A estabilidade aeróbia foi maior para silagens inoculadas com LB 45.22 e LB 90.14 aos 20 d de fermentação nos anos 1 e 2, e também para silagens inoculadas com Lalsil AS no ano 2. Com 40 e 60 d de fermentação, todas as silagens inoculadas permaneceram estáveis. As cepas de *L. buchneri* LB 45.22 e LB 90.14 mostraram consistentemente a capacidade de modular a fermentação em um curto período de armazenamento e aumentar a estabilidade aeróbia da silagem de sorgo aos 20 d de fermentação. Para o experimento 2, quatro carneiros Santa Inês x Dorper, fistuladas no rúmen, foram distribuídas em delineamento em quadrado latino 4 × 4 alimentados com as seguintes silagens como fonte de forragem: silagem sem inoculante (controle); silagem inoculada com Lalsil As; silagem inoculada com a cepa LB 50.4 e silagem inoculada com a cepa LB 90.14 (LB 90.14). Todas as silagens inoculadas e suas TMRs permaneceram estáveis ao longo de 7 d de exposição ao ar ($P < 0,001$). Não houve efeito dos inoculantes na ingestão de nutrientes ($P \geq 0,106$). As cepas de *L. buchneri* utilizadas no presente estudo foram eficientes em melhorar a estabilidade aeróbia da silagem de sorgo, sem comprometer a ingestão de nutrientes, bem como a fermentação ruminal em ovinos

alimentados com essas dietas, apresentando grande potencial para serem utilizadas como inoculantes em silagens de sorgo em condições tropicais.

Palavras-chave: Ácido acético. Deterioração aeróbia. Ingestão de MS. Levedura.

ABSTRACT

ALVES, Wagner Sousa, M.Sc., Universidade Federal de Viçosa, July, 2019. **Intake, apparent digestibility and aerobic stability of sorghum silage inoculated with different strains of *Lactobacillus buchneri***. Adviser: Odilon Gomes Pereira. Co-adviser: Karina Guimarães Ribeiro.

Two experiments were carried out to evaluate the effect of different strains of *Lactobacillus buchneri* on the aerobic stability of sorghum silage at different fermentation periods (Experiment 1) and the intake, apparent digestibility, nitrogen balance and ruminal fermentation in rams fed on sorghum silage inoculated with *L. buchneri* strains (Experiment 2). In experiment 1, sorghum was ensiled in two consecutive years after application of the following inoculants: Control; Lalsil AS; and three strains of *L. buchneri*: LB 45.22, LB 50.4, and LB 90.14. The mini silos were opened after 20, 40, and 60 d of fermentation period (FP). The lactic acid content was lower at 20 d of FP in silages inoculated with strains LB 45.22 and LB 90.14. These silages also had the highest acetic acid levels and the lowest yeast population at 20 d of fermentation. The aerobic stability was higher for LB 45.22 and LB 90.14 inoculated silages at 20 d of FP in years 1 and 2, and also for Lalsil AS inoculated silage in year 2. With 40 and 60 d of FP, all inoculated silages remained aerobically stable over the 7 d of exposure to air. *L. buchneri* strains LB 45.22 and LB 90.14 consistently showed the ability to modulate fermentation in a short period of fermentation and increase the aerobic stability of sorghum silage stored for 20 d. For experiment 2, four Santa Inês x Dorper sheep, fistulated in the rumen, were distributed in a 4 × 4 Latin square design fed the following silages as a forage source: silage without inoculant (control); silage inoculated with commercial inoculant Lalsil As; silage inoculated with strain LB 50.4 and silage inoculated with strain LB 90.14. The experimental period was 20 days. All inoculated silages and their TMRs remained stable over 7 d of exposure to air ($P < 0.001$). There was no effect of inoculants on nutrient intake ($P \geq 0.106$). The strains of *L. buchneri* used in the present study were efficient in improving the aerobic stability of sorghum silage, without compromising nutrient intake, as well as ruminal fermentation in sheep fed these diets, showing great potential to be used as inoculants in sorghum silages under tropical conditions.

Keywords: Acetic Acid. Aerobic deterioration. Intake DM. Yeasts.

SUMÁRIO

1. INTRODUÇÃO GERAL

A pecuária brasileira é altamente dependente da oferta de forragem nas pastagens, no entanto, a disponibilidade de forragem ao longo do ano é irregular, com aproximadamente 73% da produção acumulada de forragem concentrada na estação chuvosa (Pequeno et al., 2015), apresentando, além da variabilidade quantitativa, inconstância na sua qualidade nutricional, fazendo-se necessário a utilização de práticas de conservação (ensilagem ou fenação) de modo a garantir a alimentação do rebanho durante o período de escassez de forragem.

A ensilagem é o principal método de conservação de forrageiras no Brasil, sendo a principal fonte de volumoso nas dietas usadas em confinamentos de bovinos de leite ou corte (Silva et al., 2019; Silvestre e Millen, 2019). Devido a sua importância, vários estudos foram feitos visando otimizar a conservação dos nutrientes durante o processo de ensilagem até o momento da abertura dos silos (Muck et al., 2018). A técnica de ensilagem se baseia na conservação de forrageiras perenes ou anuais em condições anaeróbias, onde, bactérias do ácido lático (BAL) epifíticas ou inoculadas fermentam os carboidratos solúveis em água à ácidos orgânicos, principalmente o lático (Weinberg e Muck, 1996). A combinação de ambiente anaeróbio e pH reduzido, ocasionado pela vedação e acúmulo dos ácidos orgânicos, proporciona ambiente inapropriado para o desenvolvimento de microrganismos indesejáveis, gerando estabilidade ao material ensilado (McDonald et al., 1991; Pahlow et al., 2003).

Diversas culturas apresentam características adequadas para serem ensiladas, dentre elas, o milho destaca-se como a forrageira mais utilizada para produção de silagem no Brasil (Silva et al., 2019; Silvestre e Millen, 2019). Entretanto, em regiões semiáridas ou tropicais que apresentam má distribuição das chuvas, a cultura do sorgo (*Sorghum bicolor* (L.) Moench) assume papel importante para confecção de silagem, pois apresenta adaptações morfofisiológicas ao estresse hídrico que proporcionam alta produção de matéria seca (MS) nessas condições (Singh et al., 2014; Rakshit et al., 2017; Blum, 2017), bem como, capacidade de rebrotar após a colheita, possibilitando um segundo corte sem a necessidade de ressemeadura (Perazzo et al., 2017). Além disso, o sorgo apresenta características favoráveis ao processo de fermentação no silo, como adequado teor de carboidratos solúveis em água (maior que 80 g/kg MS), baixo poder tampão e adequado teor de MS (maior que 250 g/kg matéria natural) (McDonald et al., 1991; Rodrigues et al.,

2020). No entanto, culturas que apresentam características adequadas à ensilagem, são predispostas a produção de silagens aerobiamente instáveis (Wilkinson e Davies, 2013), devido o aumento da produção de ácido lático e baixa formação de ácido acético, comumente observado em silagens de sorgo (Tabacco et al., 2011; Fernandes et al., 2020; Diepersloot et al., 2021).

O uso de inoculantes microbianos com o intuito de melhorar o perfil fermentativo e obter silagens aerobiamente estáveis se destaca, devido a sua praticidade de aplicação. Dentre os inoculantes microbianos, destacam-se aqueles contendo cepas de *L. buchneri*, uma BAL heterofermentantiva obrigatória que metaboliza os carboidratos solúveis através da via da fosfoquetolase, resultando na produção de ácido láctico e acético (Heinl et al., 2012). Além disso, essa bactéria degrada anaerobiamente em meio ácido, o ácido lático, produzindo ácido acético, 1,2-propanodiol, CO_2 e traços de etanol (Oude Elferink et al., 2001; Heinl et al., 2012).

O aumento nas concentrações de ácido acético resulta em uma menor população de leveduras, principal microrganismo envolvido na deterioração aeróbia (Danner et al., 2003; Drouin et al 2020; Weiß et al., 2022). Como resultado, silagens expostas ao ar, inoculadas com *L. buchneri*, permanecem estáveis por mais tempo, quando comparadas às silagens não inoculadas (Arriola et al., 2021; Diepersloot et al 2021; Kung et al., 2021). No entanto, os inoculantes comerciais disponíveis no mercado requerem tempos de fermentações prolongados, para que seja observado melhorias substanciais sobre a estabilidade aeróbia (Muck et al 2018; Arriola et al., 2021), tornando-os uma escolha inadequada quando os silos são abertos com períodos curtos de armazenamento (Borreani et al., 2018; Ferrero et al., 2019).

O efeito tardio do *L. buchneri* sobre a estabilidade aeróbia pode estar associado a incapacidade dessa bactéria em competir com a microflora epifítica da planta na fase inicial de fermentação (Driehuis et al., 1999; Schmidt et al., 2009). Adicionalmente, Muck (2013) sugeriu que a origem da cepa possa estar relacionada a essa condição, pois grande parte das cepas comerciais disponíveis atualmente foram isoladas de forrageiras cultivadas em clima temperado. Dessa forma, a competitividade dessas cepas frente à microbiota epifítica e adaptação ao substrato presente é reduzida, limitando ou impedindo que as BAL inoculadas dominem a fermentação na fase inicial.

Muck et al. (2018) sugeriram que novas pesquisas devem ser feitas de modo a se identificarem novas cepas capazes de melhorar a estabilidade aeróbia em períodos curtos de armazenamento. No entanto, grande parte dos trabalhos publicados que avaliaram a estabilidade aeróbia de silagens inoculadas com novas cepas selvagens foram realizadas após longos períodos de armazenamento (Agarussi et al., 2022; Santos et al., 2013; Silva et al., 2018; Costa et al., 2021).

Avaliando novas cepas de *L. brevis* e *L. parafarraginis* isolados de silagem de milho e testadas como inoculantes na ensilagem desta cultura Xu et al. (2017) observaram que as concentrações de ácido acético e 1,2-propanodiol diferiram do controle a partir de 20 dias de ensilagem. Esses dois produtos da fermentação são os principais compostos associados à silagem aerobiamente estável (Holzer et al., 2003). Com base nesses resultados, pode-se inferir que cepas isoladas da própria cultura poderiam atuar prematuramente no processo fermentativo da silagem, promovendo uma estabilidade aeróbia satisfatória com menor tempo de armazenamento.

Maiores concentrações de ácido acético em silagens inoculadas com *L. buchneri* foram, por certo tempo associadas à redução do consumo de MS em ruminantes (Forbes et al., 1992; Anil et al., 1993). Recentemente em uma meta analise, Gerlach et al. (2021) observaram que o aumento de 1g de ácido acético por kg de MS da dieta reduz o consumo de MS em bovinos de leite em 1,2 g para cada 100 kg de peso vivo do animal. Quando o teor de ácido acético na dieta ultrapassa 17,3 g/kg de MS, a redução no consumo foi mais pronunciada, com redução de 5,6 g de consumo de MS a cada 100 kg de peso vivo a cada aumento de 1 g de ácido acético. Contudo, alguns dados utilizados nessa meta-análise foram obtidos de ensaios onde o ácido acético foi fornecido em sua forma pura e não advindo da fermentação natural em silagem, além disso, doses maiores foram utilizadas, podendo gerar um viés na interpretação dos dados. Corroborando com isso, Arriola et al. (2021) realizaram uma meta-análise do efeito da inoculação de *L. buchneri* sobre o desempenho de vacas de leite. Os autores observaram que a inoculação não reduziu o consumo de MS bem como a produção de leite.

Um estudo recente demostrou que a inoculação com BAL em silagens altera o odor da silagem (Zhang et al., 2021). Essa alteração ocorre devido à formação de compostos voláteis, perceptíveis para os animais, alterando dessa forma o odor e sabor, ponto-chave na escolha do animal ao consumir um alimento (Scherer et al.,

2019**).** No entanto, segundo Rabelo et al. (2018), o consumo de deitas preparadas com silagens inoculadas depende de vários fatores, dado que, diferentes espécies e cepas, bem como as alterações causadas na fermentação da silagem e no rúmen, podem causar diferentes impactos sobre o consumo.

Diante do exposto, sugerimos que cepas de *L. buchneri* isoladas em condições tropicais poderiam aumentar a estabilidade aeróbia da silagem em menor tempo de armazenamento. Além disso, estudos avaliando cepas selvagens como inoculantes em silagem devem ser testadas em experimentos com animais para determinar seus efeitos sobre o consumo e digestibilidade *in vivo*, assim como a fermentação ruminal e eficiência do uso de nitrogênio.

Diante do exposto, objetivou-se com esse estudo avaliar o efeito de novas cepas selvagens de *L. buchneri* isoladas em condições tropicais sobre a estabilidade aeróbia de silagem de sorgo em diferentes períodos de fermentação, bem como, o consumo, digestibilidade, fermentação ruminal e balanço de nitrogênio em cordeiros alimentados com essas silagens.

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3. CHAPTER I: Fermentation and aerobic stability of sorghum silages treated with new strains of *Lactobacillus buchneri*

Abstract: We evaluated the effect of different wild strains of *Lactobacillus buchneri* on the fermentation, chemical composition, and aerobic stability of sorghum silages at different fermentation periods (FP). Sorghum was ensiled in two consecutive years after application of the following inoculants: Control; Lalsil AS; and three strains of *L. buchneri*: LB 45.22, LB 50.4, and LB 90.14. The mini silos were opened after 20, 40, and 60 d of FP. The lactic acid content was lower at 20 d of FP in silages inoculated with strains LB 45.22 and LB 90.14 in both years. These silages also had the highest acetic acid levels and the lowest yeast population at 20 d of fermentation. The aerobic stability was higher for LB 45.22 and LB 90.14 inoculated silages at 20 d of FP in years 1 and 2, and also for Lalsil AS inoculated silage in year 2. With 40 and 60 d of FP, all inoculated silages remained aerobically stable over the 7 d of exposure to air. *L. buchneri* strains LB 45.22 and LB 90.14 consistently showed the ability to modulate fermentation in a short period of fermentation and increase the aerobic stability of sorghum silage stored for 20 d.

Keywords: acetic acid, bacterial inoculant, period of fermentation, yeast.

3.1.Introduction

The anaerobic conditions of the silo are lost after opening and diffusion of oxygen into the silage mass is inevitable. The presence of oxygen stimulates the growth of aerobic microorganisms, such as yeast, the main spoilage initiator, which oxidizes water-soluble carbohydrates (WSC) and lactic acid producing CO_2 and increasing temperature (Pahlow et al., 2003; Wilkinson & Davies, 2013). In addition, the oxidation of lactic acid results in an increase in silage pH, allowing molds and sporogenic bacteria to develop. The growth of these microorganisms in silage causes high dry matter (DM) losses and compromises the hygienic quality of the silage (Drouin et al., 2021; Tabacco et al., 2011).

Sorghum silages are prone to aerobic deterioration because their fermentation is predominantly homolactic, with low acetic acid (an antifugal agent) formation (Diepersloot et al., 2021; Fernandes et al., 2020; Filya, 2003). The use of bacterial inoculants containing *Lactobacillus buchneri* is the main strategy used to

increase the concentration of acetic acid and improve aerobic stability in silages worldwide (Muck et al., 2018). This species is an obligate heterofermentative lactic acid bacteria (LAB), capable of anaerobically degrading lactic acid into acetic acid and 1,2-propanediol (Heinl et al., 2012; Oude Elferink et al., 2001). Increased concentrations of acetic acid in silage reduce the survival and inhibit the growth of yeast (Danner et al., 2003; Weiß et al., 2022) and increasing the aerobic stability of silage (Arriola et al., 2021a).

L. buchneri-based inoculants require extended fermentation periods (FP) for satisfactory results on aerobic stability to be observed (Arriola et al., 2021a; Driehuis et al., 1999; Ferrero et al., 2019). Long FP are opposed to field conditions, since sometimes producers need to open the silo following short FP. In such cases, the use of *L. buchneri*-based inoculants could be inefficient (Muck et al., 2018). In light of this, these authors suggest that research focuses on the selection of new *L. buchneri* strains capable of dominating the microbial community and modulating fermentation early, in order to improve aerobic stability over short FP.

Several studies under tropical conditions have been conducted to select new strains, however, none have evaluated their effectiveness on aerobic stability following short FP (Agarussi et al., 2022; Costa et al., 2021; Santos et al., 2013; Silva et al., 2018). Some findings suggest that wild strains have the potential to increase aerobic stability over short FP. Eikmeyer et al. (2013), through metagenomic analysis, reported that silage inoculated with a strain of *L. buchneri* isolated from a grass silage, showed a predominance of this species after 14 d of ensiling, unlike control silage, which was dominated by *Lactobacillus plantarum* and *Lactococcus lactis*. Xu et al. (2017), evaluated a mixture of *Lactobacillus brevis* and *Lactobacillus parafarraginis* strains isolated from corn silage and tested as inoculants in this crop, and observed increased concentrations of acetic acid and 1,2-propanediol compared to the control silage from 20 d of FP.

Therefore, we hypothesized, in our study, that strains isolated from the culture itself can act more efficiently in the fermentative process of silage, resulting in satisfactory aerobic stability with a shorter FP. Thus, we aimed to evaluate the potential of different wild strains of *L. buchneri* as inoculants in sorghum silages stored at different FP and their effects on aerobic stability.

3.2. Materials and methods

Experiment location

The experiment was conducted in the Laboratory of Forage and Silage Microbiology of the Department of Animal Science of the Federal University of Viçosa (UFV), located in the city of Viçosa, Minas Gerais, Brazil. The city is located at 20° 45' south latitude and 42° 51' west longitude, with an average altitude of 657 m. The climate is of type Cwb, according to the classification proposed by Köppen, with an average annual precipitation of 1341 mm.

Experimental design

The trial was conducted according to a completely randomized design in a 5 × 3 × 2 factorial scheme (5 inoculants (Inoc), 3 FP, and 2 years (Yr)) with four replicates. The inoculants evaluated were: 1) without inoculant (Control); 2) commercial inoculant (Lalsil AS); 3) *L. buchneri*, strain LB 45.22 (LB 45.22); 4) *L. buchneri*, strain LB 50.4 (LB 50.4); and *L. buchneri*, strain LB 90.14 (LB 90.14). FP corresponded to 20, 40, and 60 d of silage storage. The crop years were 2017/2018 and 2018/2019.

The commercial inoculant used was Lalsil AS (Lallamend, Goias, Brazil), which contained the *L. buchneri* CNCM I-4323 strain. The wild strains LB 45.22, LB 50.4, and LB 90.14 were previously isolated from sorghum silages after 45, 56, and 90 d of fermentation, respectively, and belong to the microorganism collection of the Laboratory of Forage and Silage Microbiology of the Department of Animal Science – UFV. The sequences of the strains are deposited in the GenBank database with the following access numbers: *L. buchneri* 45.22 – MH936292.1; *L. buchneri* 50.4 – MH924298; and *L. buchneri* 90.14 – MH936314.1.

Cultivation, silage production, and opening of silos

Forage sorghum (*Sorghum bicolor* (L.) Moench cv. Volumax, Agroceres, Minas Gerais, Brazil) was cultivated in two agricultural years (2017/2018 and 2018/2019) in an area of 1000 m^2. In both years, the soil was prepared and sorghum sown using a row spacing of 0.8 m and 10–13 seeds per linear meter. The fertilization for planting and top dressing in the respective years were: 246 kg/ha of the NPK mixture (8-28-16) at planting and 70 kg/ha of nitrogen in top dressing (45 d after sowing), using urea as the source. The sorghum harvest was carried out

manually, with the grain in the farinaceous stage. Then, the material was chopped in a stationary forage machine (model PN Plus 2000, Nogueira S.A., São João da Boa Vista, Brazil) at a theoretical average particle size of 1.5 cm.

Sixty piles of 10 kg each (1 pile for each replication) were made for application of inoculants, in each experimental year. The Lalsil AS inoculant was rehydrated in 100 mL of distilled water and applied at an application rate of 1×10^6 colony-forming units (cfu)/g of fresh matter (FM).

Wild strains were previously prepared before ensiling. Initially, *L. buchneri* strains were cultivated in 3 mL of De Man Rogosa Sharpe (MRS) broth (Merck, Darmstadt, Germany) at 37 ºC for 16 h, and then again cultivated in 5 mL of MRS broth at 37 ºC for 16 h, because the strains were stored at −80 °C. After cultivation in 5 mL, serial dilutions were made and plated on MRS agar (Difco, São Paulo, Brazil) using sterile Petri plates, which were incubated at 37 ºC for 48 h for determination of the number of cfu/mL of MRS broth. Based on this result, the final volume of MRS broth that the strains should be grown in to reach an application rate of 1×10^6 cfu/g of FM was determined. After cultivation in the final volume for 16 h, the tubes were centrifuged at 1000 × g × 10 min and the supernatant discarded. The pellet containing the cells was diluted in 100 mL of distilled water and applied with manual sprayers. For each inoculated pile, the strains were grown individually.

Approximately 7.2 kg of the treated material was ensiled in plastic buckets of 12 L. Buckets were sealed, weighed, and stored at room temperature. The silages remained stored for 20, 40, and 60 d, and, after opening the buckets, all the material was homogenized and sampled.

Microbial population and DM recovery

An aqueous extract was obtained from 25 g of silage and 225 mL of sterile ringer's solution (Oxoid, Hampshire, England) and homogenized for 1 min in an industrial blender. The aqueous extract was filtered using a double layer of sterile gauze, and soon after, the pH was measured using a potentiometer (Tecnal, Piracicaba, Brazil).

An aliquot of the aqueous extract was submitted to serial dilutions ranging from 10^{-1} to 10^{-8} to quantify the microbial population. Plating was performed using the pour plate technique on sterile Petri plates. The LAB population was quantified in MRS agar (Difco, São Paulo, Brazil), incubated at 37 ºC for 48 h. Cultivation of

enterobacteria was performed on Violet Red Bile agar (Oxoid, Basingstoke, England), incubated at 37 °C for 24 h. Yeasts and molds were cultivated in Potato Dextrose Agar (Difco, São Paulo, Brazil) with 1.5% tartaric acid (10% vol/vol), at 25 °C for 72 and 120 h, respectively.

To estimate the DM recovery (DMR) from the silage, the buckets were weighed right after ensiling and before opening at the end of each FP. DM recovery was calculated according to Jobim et al. (2007).

Chemical analysis

Fresh forage and silage samples were dried at 55 ºC for 72 h in a forced-air oven and ground in a knife mill (Tecnal, Piracicaba, Brazil) with a 1 mm sieve. The ground samples were used to determine the DM (method 934.01), crude protein (CP; method 984.13), and acid detergent fiber (ADF; method 973.18) contents as described by the AOAC (1990). Neutral detergent fiber (NDF) concentrations were determined with the addition of α-thermostable amylase, without the use of sodium sulfite and expressed inclusive of residual ash according to Van Soest et al. (1991), modified by Senger et al. (2008) for autoclave.

A second aliquot of the aqueous extract was placed in tubes containing 100 µL of H_2SO_4 (50% vol/vol), to evaluate the concentrations of WSC (Nelson, 1944), ammonia-N (Okuda et al., 1965), and organic acids. For the quantification of organic acids, the samples were treated with calcium hydroxide and cupric sulfate and analyzed by high-performance liquid chromatography (HPLC) according to Siegfried et al. (1984). The HPLC apparatus (SPD-10 AVP, Shimadzu) was equipped with a refractive index detector and an Aminex HPX-87H column (BIO-RAD, CA, USA) with the mobile phase containing 0.005 M H_2SO_4 and a flow rate of 0.6 mL/min at 50 °C.

Aerobic stability

In all opening periods, after homogenization of the silages, a sample of 2 kg of silage was returned to its respective bucket without compaction, to assess aerobic stability. The buckets were stored in a temperature-controlled room (± 22 °C), and a data logger (iMINI temperature, Cryopak, Edison, United State) was inserted in the geometric center of the silage mass to measure the temperature every 10 min, for 7 d. Two data loggers were distributed in the room to measure the ambient temperature. Aerobic stability was defined as the number of hours the silage

remained stable before the temperature rose 2 °C above room temperature (Kung et al., 2021). At the end of the aerobic exposure period, the buckets were weighed and the material homogenized to carry out the quantification of the mold and yeast population, WSC, organic acids, and pH measurement, as described above. DM losses were calculated by the difference between the weight of the silage in the buckets before and after the 7 d of exposure to air.

Statistical analysis

Microbial population count data were transformed to a logarithmic base (log_{10} cfu/g) before statistical analysis. All data were analyzed using the SAS 9.4 GLM procedure (SAS System Inc., Cary, NC, USA). Inoculants, FP, years, and their interactions were considered fixed effects in the model.

The homogeneity of variances between treatments was assumed and variables related to fermentation profile, microbial population, and chemical composition were subjected to analysis of variance and means were compared by Tukey's test, considering 0.05 as a critical level for the occurrence of a type error I. When the Inoc × FP × Yr interaction was significant, the variables were split by fixing the year effect.

3.3. Results

The DM of the sorghum plant before ensiling were 259.4 and 248.4 g/kg of FM in the years 1 and 2, respectively (Table 1). The WSC concentration were 169.5 and 239.1 g/kg of DM in the years 1 and 2. The population of LAB, enterobacteria, yeast, and mold was 6.24, 7.48, 6.03 and 6.40 and 7.1, 7.4, 5.94 and 5.6 log_{10} cfu/g of FM in the years 1, and 2, respectively (Table 1).

Table 2 shows the p-values and standard error of mean (SEM) of the fermentation profile, microbial population, and chemical composition of the silages. There was an effect of the Inoc × FP × Yr interaction for most variables ($P \leq 0.029$), except for DM, ADF, CP, and DMR, which were affected only by FP ($P \leq 0.048$). The splitting of inoculants and fermentation periods were carried out within each experimental year.

The pH values were higher at 20 d of FP for the control silage and that inoculated with LB 45.22 in year 1 (Table 3). As the FP progressed, the pH of control and Lalsil AS inoculated silages decreased, however, there was no difference

between treatments with 40 and 60 d of FP. In year 2, at 20 d of FP, there was no effect of inoculants on pH. At 40, the control silage and those inoculated with LB 50.4 had the lowest pH values. With 60 d of FP, the silage inoculated with LB 50.4 had the lowest pH (Table 3).

The lactic acid contents were higher for the control and inoculated silages with Lalsil AS and LB 50.4 in all FP, except at 60 d for silage inoculated with LB 50.4 in year 1. In year 2, the control and silages inoculated with LB 50.4 had the highest lactic acid contents in all FP. At 20 d of FP, the silages inoculated with LB 45.22 and LB 90.14 presented the lowest contents of lactic acid (Table 3).

In year 1, acetic acid contents were higher for silages inoculated with LB 45.22 and LB 90.14 in all FP (Table 3). In year 2, all inoculated silages had higher acetic acid contents compared to the control silage, except for that inoculated silage with LB 50.4 at 20 d of FP (Table 3).

Ethanol concentrations were similar among silages at 20 d of FP in year 1 (Table 3). However, at 40 d, the control silage and those inoculated with LB 45.22 and LB 90.14 showed the lowest concentrations. With 60 d of FP, the control silage had the highest ethanol content. In year 2, ethanol concentrations differed among inoculants only at 40 d of FP (Table 3).

In year 1, ammonia-N contents differed among inoculants only at 60 d of FP, with lower values being registered in silages treated with wild strains (Table 3). In year 2, the control silage showed the highest levels of ammonia-N at 20 and 60 d of FP (Table 3).

A higher concentration of WSC was observed in the silage treated with Lalsil AS at 60 d of FP in year 1, while in year 2, a higher concentration of WSC was recorded at 40 and 60 d of FP in the control silage (Table 3).

Table 4 shows the microbial populations and the NDF contents of the silages. In both years evaluated, the population of BAL was higher in the inoculated silages in all FP compared to the control silage (Table 4).

Mold counts did not differ between inoculants at all FP in year 1 (Table 4). However, their count was higher for the control silage compared to the inoculated silages at 20 and 40 d of FP in year 2.

The yeast population was lower in the silages inoculated with LB 45.22 and LB 90.14 at 20 d of FP in year 1 (Table 4). In year 2, the yeast population was lower in the silage inoculated with Lalsil AS at 20 d of FP. In both years, at 40 and 60 d of

FP, all inoculated silages had a lower yeast population compared to the control silage (Table 4).

Silage inoculated with LB 90.14 presented a lower NDF content at 40 d of FP compared to the control silage and that inoculated with LB 50.4, in year 1 (Table 4). In year 2, silage inoculated with Lalsil AS had a lower NDF content at 60 d of FP compared to that treated with LB 50.4.

The DM content reduced from 242.8 g/kg FM at 20 d of FP to 234.5 g/kg FM at 60 d of FP (Figure 1A). The DMR was lower after 60 d of FP, registering a value of 910.8 g/kg DM (Figure 1B). The CP (Figure 1C) and ADF (Figure 1D) contents of the silages increased from 73.3 and 309.6 g/kg DM at 20 d of FP to 76 and 321.5 g/kg DM at 60 d of FP, respectively.

Table 5 shows the p-values and SEM of the variables analyzed at the end of the 7 d of aerobic exposure. There was an Inoc × FP × Yr interaction effect for all variables ($P \leq 0.046$).

In year 1, at 20 d of FP, silages inoculated with LB 45.22 and LB 90.14 remained stable after 7 d of exposure to air (> 168 h). The control silage and those inoculated with Lalsil AS and LB 50.4 lost the aerobic stability after ± 51.83 h of exposure to air. All inoculated silages remained stable throughout the 7 d exposure (> 168h) after 40 and 60 d of FP, while the control silage lost its stability after 57.5 and 94.75 h of exposure to air, respectively (Figure 2A).

In year 2, the silages stored for 20 d and inoculated with Lalsil AS, LB 45.22, and LB 90.14 remained stable throughout the 7 d of aerobic exposure (> 168 h). With 40 and 60 d of FP, all inoculated silages remained stable throughout the 168 h of exposure to air. The control silage lost its stability at 35.46, 37.38, and 59.78 h after exposure to air at 20, 40, and 60 d of FP, respectively (Figure 2B).

In year 1, the control silage and that inoculated with Lalsil AS and LB 50.4 reached the highest maximum temperatures over the 7 d of aerobic exposure, at 20 d of FP, while the silages inoculated with LB 45.22 and LB 90.14 maintained their temperature close to room temperature (Table 6). The control silage reached the highest maximum temperature at 40 and 60 d of FP, reaching 34.78 and 29.75 ºC over the 7 d of aerobic exposure, respectively. In year 2, the control silage reached the highest maximum temperature throughout the 7 d of aerobic exposure, regardless of the FP. The silages inoculated with Lalsil AS, LB 45.22, and LB 90.14, with 20 d of FP, presented the lowest maximum temperatures (Table 6). Sorghum

silage temperatures over 7 d of aerobic exposure are shown in supporting figures S1 (year1) and S2 (year2).

Silages inoculated with LB 45.22 and LB 90.14 with 20 d of FP had lower pH values at the end of the 7 d of aerobic exposure, while the control silage had the highest pH values, independently of the FP, in year 1 (Table 6). In year 2, the silages inoculated with Lalsil AS, LB 45.22, and LB 90.14, with 20 d of FP, presented the lowest pH values at the end of the aerobic stability evaluation (Table 6).

The silages inoculated with LB 45.22 and LB 90.14 in year 1, with 20 d of FP, had the lowest yeast populations at the end of the 7 d of aerobic exposure, while in year 2, at 20 d of FP, the yeast population was lower for silage inoculated with LB 90.14 (Table 6). The control silage had the highest yeast counts in all fermentation periods, except at 20 d, when the silage inoculated with LB 50.4 had similar yeast counts (Table 6).

The mold population, after 7 d of aerobic exposure, was lower in the silage inoculated with LB 90.14 at 20 d of FP in year 1 (4.12 log cfu/g FM). At 40 and 60 of FP, all inoculated silages had a lower mold population compared to the control silage after aerobic exposure. For year 2, all inoculated silages had a lower mold population compared to the control silage after 7 d of exposure to air, regardless of FP (Table 6).

The silage inoculated with LB 90.14, in year 1, at 20 d of FP, presented a higher lactic acid content (53.3 g/kg of DM) at the end of the 7 d of aerobic exposure, while the control silage and that inoculated with Lalsil AS and LB 50.4 had levels below detection levels. In year 2, the concentration of lactic acid was lower in the control silage and that inoculated with LB 50.4 after 7 d of aerobic exposure in the silages stored for 20 d, whereas at 40 and 60 d of FP, all inoculated silages had higher concentrations of lactic acid in relation to the control silage at the end of the 7 d of aerobic exposure (Table 6).

The acetic acid content was higher at the end of the 7 d of aerobic exposure for silage inoculated with LB 90.14 to 20 d of FP in year 1. In year 2, the silages inoculated with Lalsil AS, LB 45.22, and LB 90.14, stored for 20 d, showed the highest levels of acetic acid (Table 6). In the control silage, in both years of evaluation, acetic acid contents were below detection levels in all FP (Table 6).

Ethanol contents in year 1 at the end of the 7 d of aerobic exposure were similar between inoculants with 20 and 60 d of FP, while in year 2, with 20 d of FP, all silages had similar ethanol contents (Table 6).

The WSC contents of the silages with 20 d of FP at the end of the 7 d of aerobic exposure in year 1 were higher in the silages inoculated with LB 45.22 and LB 90.14. In year 2, at 20 d of FP, the silages inoculated with Lalsil AS, LB 45.22, and LB 90.14 showed the highest levels of WSC after 7 d of exposure to air (Table 6).

The DM loss of silages stored for 20 d, after 7 d of exposure to air, in year 1, was less for silages inoculated with LB 45.22 and LB 90.14. At 40 d of FP, the inoculated silages showed a smaller DM loss compared to the control silage. In year 2, the control silage showed a higher DM loss compared to the inoculated silages, regardless of the FP (Table 6).

3.4. Discussion

The events that take place right after the silo is sealed are highly complex and dependent on the characteristics of the plant at the time of harvest (Pahlow et al., 2003). Among these characteristics, the DM and WSC contents directly influence the final quality of the silage. In our study, plant DM contents at the time of ensiling were close to the minimum 250 g/kg of FM contents suggested by McDonald et al. (1991) and WSC contents above 125.4 g/kg of DM suggested by (Rodrigues et al., 2020) for sorghum plants. The chemical composition of the sorghum plant before ensiling was similar to that reported in the literature under tropical conditions (dos Anjos et al., 2018; Filya, 2003; Rodrigues et al., 2020).

In the present study, inoculation had inexpressive effect on chemical composition and DM recovery, corroborating Agarussi et al. (2022) and Kung et al. (2021). Due to its obligatory heterofermentative metabolism, it is assumed that inoculation with *L. buchneri* would increase DM losses due to CO_2 formation through WSC fermentation and anaerobic degradation of lactic acid (Oude Elferink et al., 2001). However, this hypothesis does not seem to apply to our study. In fact, in a recent meta-analysis, (Arriola et al., 2021a) observed that inoculation of sorghum silage with *L. buchneri* did not increase DM losses. On the other hand, the lower DMR at 60 d of FP can be attributed to the longer period of fermentation that these silages experienced.

The inoculation showed a great variation in the response among the studied strains in function of the evaluated FP and these variations were more evident at 20 d. The lower lactic acid content of the silages inoculated with LB 45.22 and LB 90.14 at 20 d of FP can be attributed to the degradation of lactic acid to acetic acid and 1,2 propanediol (Oude Elferink et al., 2001), evidenced by the higher contents of acetic acid in these silages at 20 d of FP in years 1 and 2, and also for the silage inoculated with Lalsil AS in year 2. However, the pH of these silages was below 4.2, considered adequate for conservation of the ensiled mass (Kung et al., 2018).

The mechanism of activation of the lactic acid to acetic acid degradation pathway by *L. buchneri* remains unclear. However, the literature emphasizes that the anaerobic degradation of lactic acid only occurs at an acidic pH (< 4.3) and when a large part of the WSC is depleted (Johanningsmeier & McFeeters, 2013; Oude Elferink et al., 2001). In the two years of evaluation, all silages presented a pH below 4.3 from 20 d of FP and WSC contents numerically smaller for LB 45.22 and 90.14 in year 1. In year 2, WSC contents were lower in silages inoculated with Lalsil AS, LB 45.22, and LB 90.14. The combination of these two factors may have triggered the lactic acid degradation pathway, modulating fermentation in the early stages and increasing acetic acid contents in the shortest period of fermentation evaluated. In general, the onset of lactic acid degradation only occurs after 28 d of fermentation, when significant increases in 1,2 propanediol are observed (Diepersloot et al., 2021; Oude Elferink et al., 2001; Santos et al., 2016). However, the ability to activate the lactic acid degradation pathway into acetic acid is variable and dependent on the strain used (Daughtry et al., 2018). According to Carvalho et al. (2021), the existence of accessory DNA in prokaryotes may explain the different responses observed when several strains of the same species are evaluated. Accessory DNA can encode new biochemical pathways and functions that confer advantages, such as, for example, adaptation to different ecological niches and the ability to compete with the epiphytic microbiota (Carvalho et al., 2021), which may explain the different responses of the evaluated strains.

There is a concern about the negative effect of increasing levels of acetic acid in inoculated silages on voluntary intake by ruminants. In a recent meta-analysis, Gerlach et al. (2021) observed that the increase of 1 g of acetic acid per kg of DM from the TMR reduced voluntary intake by 1.2 g (per 100 kg BW) in dairy cows. When the acetic acid content exceeded 17.3 g/kg DM in the TMR, the

reduction in voluntary intake was 5.6 g for every further 1 g of acetic acid in the TMR. However, some data used in this meta-analysis come from experiments where acetic acid was supplied in its pure form and in doses higher than those commonly found in silage, which may generate a bias. Corroborating this, Arriola et al. (2021a) performed a meta-analysis on the impact of inoculation with *L. buchneri* on the performance of dairy cows. The authors observed that inoculation did not affect DM intake as well as milk yield. In a study carried out in our laboratory, using the same strains as in this study (except for the LB 45.22 strain), we did not observe a reduction in consumption in lambs fed with inoculated sorghum silage in relation to the control silage (unpublished data).

All inoculated silages showed higher LAB counts compared to the control silage in all FP, indicating that the tested strains were able to compete with the epiphytic population and dominate the bacterial population in the initial fermentation phase. Even with similar LAB populations in all fermentation periods, strains LB 45.22 and LB 90.14 differed in their ability to modulate fermentation in the initial FP, as shown above. The results corroborate those of (Daughtry et al., 2018), who observed that the growth rate of *L. buchneri* does not correlate with the strain's ability to metabolize lactic acid into acetic acid, being a specific characteristic of each strain.

The lower yeast population in the silages inoculated with LB 45.22 and LB 90.14 (years 1 and 2) and Lalsil AS (year 2) at 20 d of FP, as well as in silages treated at 40 and 60 d of FP, can be attributed to the higher acetic acid concentration in these silages, as suggested by Danner et al. (2003). Acetic acid has an antifungal action, capable of reducing the survival of yeasts during fermentation and inhibiting their growth when the silage is exposed to air (Driehuis et al., 1999; L. Kung et al., 2021). Acetic acid has a higher pKa than lactic acid and is in its non-dissociated form, which is permeable in the plasma membrane of yeasts, which dissociates upon reaching the intracellular medium, releasing protons and acidifying the cytoplasm, leading the yeasts to energy exhaustion due to the energy cost of expelling protons to the external environment (Moon, 1983; Stratford et al., 2013).

The reduction in the yeast population due to the increase in acetic acid contents in the silages inoculated with LB 45.22 and LB 90.14 (years 1 and 2) and Lalsil AS (year 2) resulted in greater aerobic stability of these silages at 20 d of FP (> 168 h). Yeasts are the main microorganisms responsible for initiating the aerobic deterioration process, as some species are acid tolerant, capable of metabolizing

lactic acid under aerobic conditions (Drouin et al., 2021; Kung et al., 2021). In addition to yeasts, bacteria of the *Acetobacter* genus can also initiate the silage deterioration process (Spoelstra et al., 1988). In our study, yeasts were probably responsible for initiating the deterioration process, as observed by the expressive increase in yeast counts at the end of the 7 d of exposure to air in the control silage and that inoculated with LB 50.4 (years 1 and 2) and Lalsil AS (year 1) with 20 d of FP and in the control silage with 40 and 60 d of FP.

We did not find studies demonstrating significant improvements in the aerobic stability of silages treated with *L. buchneri* in a fermentation period less than or equal to 20 d. However, improvements in aerobic stability are reported over prolonged periods of fermentation (Arriola et al., 2021a; Diepersloot et al., 2021; Driehuis et al., 1999). Diepersloot et al. (2021) observed that sorghum silage inoculated with a mixture of *L. plantarum*, *L. buchneri*, and *Lactobacillus diolivorans* increased by 47 and 156 h more in aerobic stability compared to the control silage only after 28 and 56 d of fermentation, respectively, while at 14 d, there was no difference between treatments. The literature emphasizes that inoculation with *L. buchneri* requires that the silage be stored for at least 45 d in order to observe the classic response to inoculation with this species (Muck et al., 2018). However, Xu et al. (2020) highlighted that the fermentation time to observe the response to inoculation with *L. buchneri* is variable and depends on the characteristics of the plant at the time of ensiling.

In a recent meta-analysis study, Arriola et al., (2021) observed that inoculation with *L. buchneri* improved aerobic stability at all intervals of fermentation periods studied (from 7 to 30, 31 to 60, 61 to 90, and greater than 90 d of fermentation), however, only after 90 d of fermentation were the inoculation effects greater and more expressive (100 h more aerobic stability than the control silage). Comparing our data with that of this meta-analysis, we observed that after 20 d of fermentation, the silages inoculated with LB 45.22 and 90.14 (year 1 and 2) and Lalsil AS (year 2) showed similar effects (132 h more aerobic stability than control silage) to that reported by Arriola et al. (2021a).

A new species of obligatory heterofermentative LAB, *Lactobacillus hilgardii*, is being studied extensively, with the hypothesis that it may improve stability in short fermentation periods. However, data in the literature are inconclusive, presenting

results similar to *L. buchneri* (Arriola et al., 2021b; da Silva et al., 2021; Ferrero et al., 2019).

The increase in the pH of deteriorated silages is a result of the oxidation of lactic acid by yeasts, which were close to the minimum detection level in our study. The silages that did not deteriorate over the 7 d of aerobic exposure had pH and organic acid contents similar to the silage at the time of opening the silos. A similar response was observed by Drouin et al. (2021) on corn silage inoculated with *L. buchneri* + *L. hilgardii*. These authors recorded pH and concentrations of organic acids after 10 d of exposure to air similar to those observed in inoculated silage at the time of opening the silos, while the control silage had a final pH of 5.47 and 0.52% of lactic acid, similar to that observed in our study.

The silages that lost their aerobic stability had the highest DM loss and the maximum temperatures reached at the end of the 7 d of aerobic exposure. The metabolic activity of spoilage microorganisms results in an increase in the temperature of the silage mass (Okatsu et al., 2019; Pahlow et al., 2003), being the main indicator of the beginning of the deterioration process (Shan et al., 2021). The highest temperature peaks reached are due to the larger population of yeasts and molds in these silages, which start to consume WSC and organic acids, forming CO_2 and increasing DM losses (Wilkinson & Davies, 2013). In addition, deteriorated silages have lower DM digestibility and metabolizable energy concentrations (Auerbach & Nadeau, 2020), as well as lower voluntary intake due to organoleptic factors (Brüning et al., 2018; Gerlach et al., 2013). Furthermore, the increased mold population can lead to the formation of mycotoxins, affecting the hygienic quality of the silage and animal health (Drouin et al., 2021; Reisinger et al., 2019).

3.5. Conclusions

In conclusion, strains LB 45.22 and LB 90.14 consistently showed the ability to modulate fermentation in a short period of fermentation and increase the aerobic stability of sorghum silage stored for 20 d, indicating potential for use as an inoculant for sorghum silage in tropical conditions. However, studies are needed to assess the capacity of these strains in commercial scale silos, as well as involving animals.

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Table 1. Chemical composition (g/kg of DM), microbial population (log_{10} cfu/g of FM) and pH of sorghum forage before ensiling.

Items¶	Year 1	Year 2
DM (g/kg of FM)	259.4	248.4
CP	63.2	72.4
NDF	563.6	568.8
WSC	169.5	239.1
LAB	6.24	7.10
Enterobacteria	7.48	7.40
Yeast	6.03	5.94
Mold	6.40	5.60
pH	5.41	5.73

¶ DM = dry matter, CP = crude protein, NDF = neutral detergent fiber, WSC = water-soluble carbohydrates, LAB = lactic acid bacteria.

Table 2. P-value and standard error of mean of the fermentation profile variables, microbial population and chemical composition of sorghum silage inoculated with different strains of *Lactobacillus buchneri* at different fermentation periods.

	Probability[#]						
Item[¶]	Inoc	FP	Yr	Inoc×FP	FP×Yr	Inoc×FP×Yr	SEM[§]
pH	0.396	0.302	<0.001	0.126	0.063	0.005	0.010
Lactic acid	<0.001	0.928	<0.001	<0.001	0.787	<0.001	0.220
Acetic acid	<0.001	<0.001	<0.001	<0.001	0.475	<0.001	0.181
Ethanol	<0.001	0.013	<0.001	<0.001	0.429	0.029	0.053
Ammonia-N	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	0.181
WSC	<0.001	<0.001	0.001	<0.001	0.847	<0.001	0.251
LAB	<0.001	<0.001	<0.001	0.594	0.060	0.002	0.052
Mold	<0.001	0.566	0.159	0.042	<0.001	0.020	0.065
Yeast	<0.001	<0.001	<0.001	0.015	<0.001	<0.001	0.127
DM	0.079	<0.001	<0.001	0.748	0.062	0.161	0.084
NDF	0.062	0.002	0.614	0.954	0.066	0.007	0.206
ADF	0.192	0.001	0.085	0.496	0.416	0.072	0.134
CP	0.491	0.048	0.040	0.740	0.360	0.694	0.044
DMR	0.209	0.001	0.001	0.435	0.035	0.565	0.328

[¶] WSC = water-soluble carbohydrates, LAB = lactic acid bacteria, DM = dry matter, NDF = neutral detergent fiber, ADF = acid detergent fiber, CP = crude protein, DMR = DM recovery.

[#] Probability of an Inoc = inoculant, FP = fermentation period, Yr = year, Inoc × FP = interaction inoculant with fermentation period, FP × Yr = interaction fermentation period with year, Inoc × FP × Yr = interaction inoculant with fermentation period and year effects.

[§] SEM = standard error of mean.

Table 3. The pH and fermentation profile (g/kg of DM basis unless stated otherwise) of sorghum silages treated with different strains of *Lactobacillus buchneri* at different fermentation periods.

	Year 1			Year 2		
	Fermentation periods (d)					
Inoculant	20	40	60	20	40	60
			pH			
Control	3.58Aa	3.53Ab	3.45Ac	3.56Aa	3.57Ba	3.56Aba
Lalsil AS	3.48Ba	3.45Aab	3.37Ab	3.53Ac	3.67Aa	3.59Ab
LB 45.22	3.55Aa	3.53Aa	3.49Aa	3.56Ab	3.67Aa	3.62Aa
LB 50.4	3.47Ba	3.47Aa	3.47Aa	3.52Aa	3.55Ba	3.51Ba
LB 90.14	3.49Ba	3.50Aa	3.48Aa	3.56Ab	3.68Aa	3.61Ab
			Lactic acid			
Control	96Ab	101.4Ab	127.3Aa	67.6Aa	68.1Aa	70.3Aa
Lalsil AS	100.5Aa	93.5ABa	110.8Aa	48.7Ba	42.9Ba	43.7Ba
LB 45.22	74.1Ba	69.6Cab	59.2Bb	41.7Ca	42.4Ba	45.5Ba
LB 50.4	96.4Aa	95.6ABa	77.6Ba	67.0Aa	67.1Aa	72.3Aa
LB 90.14	73.6Bab	76.6BCa	61.1Bb	41.6Ca	45.3Ba	41.0Ba
			Acetic acid			
Control	3.4Cb	4.6Cab	8.3Ca	1.3Ba	1.6Ba	3.1Ba
Lalsil AS	11.1BCb	21.3Bb	33.3Ba	34.4Ab	41.9Aab	49.6Aa
LB 45.22	21.8ABb	45.8Aa	57.2Aa	27.9Ab	41.5Aab	50.1Aa
LB 50.4	4.4Cc	17.9Bb	40.8ABa	7.0Bc	46.5Ab	57.1Aa
LB 90.14	35.2Ab	52.9Aa	54.6Aa	33.4Ab	39.5Aab	46.9Aa
			Ethanol			
Control	10.3Ab	9.4Bb	19.4Aa	4.3Ab	10.5Aa	6.7Aab
Lalsil AS	6.5Ab	14.1ABa	11BCab	5.6Aa	5.9Ba	2.6Aa
LB 45.22	16.4Aa	8.0Ba	5.0CDa	10.3Aa	7.8ABab	3.8Ab
LB 50.4	14.8Aa	19.1Aa	11.2Ba	11.1Aa	8.3ABa	5.5Aa
LB 90.14	10.5Aa	7.6Ba	4.7Da	5.4Aab	7.0ABa	2.0Ab
			Ammonia-N, g/kg of total N			
Control	91.6Ab	83.1Ab	109.2Aa	52.0Aa	49.7Aa	63.1Aa
Lalsil AS	83.6Aa	90.2Aa	84.3Ba	43.1ABb	54.1Aa	58.7Aba
LB 45.22	79.5Aa	86.1Aa	59.1Cb	40.7Aba	47.3Aa	47.7Ca
LB 50.4	79.8Aa	83.6Aa	63.9Ca	45.5Aba	53.6Aa	50.9BCa
LB 90.14	76.0Ab	85.9Aa	64.2Cc	36.2Bb	45.6Aab	53.1ABC
			WSC[#]			
Control	48.1Aa	20.8Aa	17.2ABa	113.3Aa	67.0Ab	65.4Ab
Lalsil AS	33.2Aa	21.4Aa	20.5Aa	11.5Ca	9.0Ba	11.6Ba
LB 45.22	13.1Aa	14.7Aa	14.6ABa	9.1Ca	8.9Ba	9.9Ba
LB 50.4	58.0Aa	16.0Ab	14.3Bb	72.4Ba	28.0Bab	18.2Bb
LB 90.14	12.7Aa	15.2Aa	13.9Ba	8.5Cb	9.1Bab	12.1Ba

[a-c]Means followed by the same lowercase letter in the row are not different according to Tukey's test ($P > 0.05$).

[A-D] Means followed by the same uppercase letter in the columns are not different according to the Tukey's test ($P > 0.05$).

[¶] Control = no inoculant, Lalsil AS = commercial inoculant containing *L. buchneri* CNCM I-4323, LB 45.22 = *L. buchneri*; strain 45.22; LB 50.4 = *L. buchneri*; strain 50.4, LB 90.14 = *L. buchneri*; strain 90.14.

[#] WSC = water-soluble carbohydrates

Table 4. Microbial population (log_{10} cfu/g of FM) and NDF (g/kg of DM) of sorghum silage treated with different strains of *Lactobacillus buchneri* at different fermentation periods.

	Year 1			Year 2		
	Fermentation periods (d)					
Inoculant[¶]	20	40	60	20	40	60
			LAB[#]			
Control	8.46Ba	8.39Ba	7.73Ca	8.37Ba	8.44Ba	7.98Ba
Lalsil AS	9.62Aa	9.61Aa	8.89Bb	9.41Aa	9.23Aab	8.89Ab
LB 45.22	9.58Aa	9.57Aa	9.14ABb	9.28Aa	9.01Aa	8.56ABb
LB 50.4	9.52Aa	9.61Aa	8.85ABb	9.31Aa	9.48Aa	8.91Ab
LB 90.14	9.69Aab	9.84Aa	9.26Ab	9.32Aa	9.0Ab	8.92Ab
			Mold			
Control	2.16Ab	3.39Aa	2.77Aab	3.47Aa	3.02Aa	2.81Aa
Lalsil AS	2.69Aa	2.83Aa	2.60Aa	2.75Aba	1.77ABC	2.33Aa
LB 45.22	ND[§]	2.63Aa	2.88Aa	2.30Ba	1.30Cb	2.03Aa
LB 50.4	2.20Aa	2.70Aa	3.02Aa	2.99Aba	1.56BCb	2.41Aab
LB 90.14	ND	2.29Aa	2.51Aa	2.21Ba	2.79ABa	2.15Aa
			Yeast			
Control	7.04Aa	6.75Aa	5.69Ab	5.81Aa	6.05Aa	5.49Aa
Lalsil AS	6.01Aa	3.91Cb	3.48BCb	2.72Ca	3.13Ba	3.32Ba
LB 45.22	3.84Ba	3.08CDa	2.91BCa	3.68BCa	3.24Ba	3.03Ba
LB 50.4	5.67Aa	5.19Ba	3.83Bb	4.81Aba	3.31Bb	3.17Bb
LB 90.14	3.40Ba	2.66Db	2.84Cab	3.47BCa	3.28Ba	3.27Ba
			NDF[¥]			
Control	574.2Aa	579.8Aa	589.8Aa	543.5Aa	556.0Aa	569.3Ab
Lalsil AS	542.4Aa	569.9Ab	563.7Aa	568.2Aa	548.0Aa	559.6Ba
LB 45.22	555.4Aa	556.3Ab	560.9Aa	568.2Aa	561.7Aa	588.6Ab
LB 50.4	567.7Aa	589.6Aa	574.7Aa	561.2Aa	544.8Ab	595.5Aa
LB 90.14	544.6Aa	544.3Ba	569.0Aa	562.9Aa	559.3Aa	567.8Ab

[a-b]Means followed by the same lowercase letter in the row are not different according to Tukey's test ($P > 0.05$).

[A-D]Means followed by same uppercase letters in the columns are not different according to the Tukey's test ($P > 0.05$).

[¶] Control = no inoculant, Lalsil AS = commercial inoculant containing *L. buchneri* CNCM I-4323, LB 45.22 = *L. buchneri;* strain 45.22, LB 50.4 = *L. buchneri*; strain 50.4, LB 90.14 = *L. buchneri*; strain 90.14.

[#] LAB = Lactic acid bacteria.

[§] ND = not detected

[¥] NDF = neutral detergent fiber.

Table 5. P -value and standard error of mean of the aerobic stability, maximum temperature reached, pH, yeast, mold, organic acids, water-soluble carbohydrates and DM loss of sorghum silage treated with different strains of *Lactobacillus buchneri*, after 7 d of aerobic exposure.

	Probability[#]						
Item[¶]	Inoc	FP	Yr	Inoc×FP	FP×Yr	Inoc×FP×Yr	SEM[§]
Aerobic	<0.001	<0.001	0.010	<0.001	<0.001	<0.001	4.777
MTR	<0.001	<0.001	0.020	<0.001	<0.001	<0.001	0.589
pH	<0.001	<0.001	0.027	<0.001	<0.001	<0.001	0.092
Yeast	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.214
Mold	<0.001	<0.001	<0.001	0.001	<0.001	0.046	0.179
Lactic acid	<0.001	<0.001	0.271	<0.001	<0.001	<0.001	0.242
Acetic acid	<0.001	<0.001	<0.001	<0.001	0.894	<0.001	0.183
Ethanol	<0.001	<0.001	0.950	0.111	0.002	0.008	0.020
WSC	<0.001	0.044	<0.001	<0.001	0.002	<0.001	0.082
DM loss	<0.001	<0.001	0.022	<0.001	0.142	<0.001	0.543

[¶] MTR = Maximum temperature reached, WSC = water-soluble carbohydrates.

[#] Probability of an Inoc = inoculant, FP = fermentation period, Yr = year, Inoc × FP = interaction inoculant with fermentation period, FP × Yr = interaction fermentation period with year, Inoc × FP × Yr = interaction inoculant with fermentation period and year effects.

[§] SEM = standard error of mean.

Table 6. Maximum temperature reached (ºC), pH, mold and yeast population (log_{10} cfu/g of FM), fermentation profile (g/kg of DM) and DM losses (g/kg of DM) of sorghum silages treated with different strains of *Lactobacillus buchneri*, after 7 d of aerobic exposure.

	Year 1			Year 2		
	Fermentation periods (d)					
Inoculant¶	20	40	60	20	40	60
			MTR#			
Control	36.80Aa	34.78Aab	29.75Ab	38.03Aa	38.23Ba	34.47Aa
Lalsil AS	34.88Aa	20.70Bb	20.43Bb	22.80Ca	22.48Ab	22.13Bc
LB 45.22	23.10Ba	20.68Bb	20.40Bb	22.75Ca	22.48Ab	22.17Bc
LB 50.4	33.38Aa	20.70Bb	20.48Bb	33.50Ba	22.60Ab	22.13Bb
LB 90.14	22.65Ba	20.55Bb	20.45Bb	22.68Ca	22.58Ab	22.20Bb
			pH			
Control	6.84Aa	5.73Ab	4.43Ac	5.44Aa	5.56Aa	5.77Aa
Lalsil AS	5.65Ba	3.48Bb	3.46Bb	3.60Ba	3.65Ba	3.62Ba
LB 45.22	3.68Ca	3.57Ba	3.52Ba	3.64Ba	3.64Ba	3.62Ba
LB 50.4	5.38Ba	3.55Bb	3.57Bb	5.00Aa	3.53Bb	3.53Bb
LB 90.14	3.53Ca	3.54Ba	3.59Ba	3.62Ba	3.65Ba	3.62Ba
			Yeast			
Control	9.24Aa	9.58Aa	9.26Aa	9.22Aa	9.29Aa	9.12Aa
Lalsil AS	9.14Aa	6.67Bb	3.58Cc	5.37Ba	4.46Ba	5.01Ba
LB 45.22	5.44Ba	3.95Db	4.14Cb	5.44Ba	3.52Bb	3.45Cb
LB 50.4	8.98Aa	4.99Cb	5.24Bb	9.08Aa	4.36Bb	4.14BCb
LB 90.14	5.01Ba	3.74Db	3.44Cb	4.51Ca	3.77Ba	3.63Ca
			Mold			
Control	7.66Aa	6.92Aab	5.89Ab	6.32Aa	6.06Aa	6.30Aa
Lalsil AS	6.81Aba	4.27Bb	2.61Bb	3.55Ba	2.25Bb	2.88Bab
LB 45.22	4.93BCa	3.05Bab	2.45Bb	3.19Ba	1.63Bb	2.01Bb
LB 50.4	7.08Aba	3.20Bb	2.77Bb	4.10Ba	2.00Bb	2.42Bb
LB 90.14	4.12Ca	2.85Bb	2.76Bb	3.00Ba	2.09Bb	2.28Bab
			Lactic acid			
Control	ND§	ND	24.4Ca	1.2Ba	ND	4.4Ba
Lalsil AS	ND	77.1Ab	89.1Aa	54.9Aa	42.9Aa	45.3Aa
LB 45.22	26.8Ba	51.3Ba	49.2CBa	47.8Aa	52.4Aa	44.8Aa
LB 50.4	1.6Cc	48.9Bb	65.5ABa	14.6Bb	54.8Aa	46.9Aab
LB 90.14	53.3Aa	60.7Ba	49.2CBa	47.1Ab	52.6Aab	56.8Aa
			Acetic acid			
Control	ND	ND	ND	ND	ND	ND
Lalsil AS	ND	19.2Ba	23.5Ba	39.4Aa	42.5ABa	47.6Aba
LB 45.22	13.4Bb	37Aa	37.5Aa	37.1Aa	47.7Aa	48.3Aa
LB 50.4	ND	12.5BCb	29.6ABa	5.4Bb	33.7Ba	30.8Ba
LB 90.14	37.1Aa	39.3Aa	37.4Aa	35.8Ab	50.4Aa	61.9Aa
			Ethanol			
Control	0.2Aa	0.7Ca	2.6Aa	1.0Aa	1.5Ba	0.4Ba
Lalsil AS	1.7Ab	7.1Aa	3.4Ab	2.5Aab	4.1Aa	1.8Ab
LB 45.22	2.7Aa	3.5BCa	2.7Aa	3.2Aa	4.3Aa	1.6Ab

LB 50.4	0.5Ab	0.56ABa	0.43Aa	5.3Aa	4.4Aa	1.9Aa
LB 90.14	1.6Aa	1.8Ca	1.9Aa	2.4Ab	4.8Aa	1.3ABb
			WSC[¥]			
Control	7.1Bab	6.1Bb	8.5Ca	5.6Ba	5.6Ba	5.6Ba
Lalsil AS	6.6Bb	31Aa	25.1Aa	22.5Aa	14.5ABb	15.2Ab
LB 45.22	26.3Aa	28Aa	19.6ABa	23.3Aa	15ABb	14Ab
LB 50.4	7.4Ba	12.4Ba	13.6BCa	8.1Bb	19.2Aab	24.1Aa
LB 90.14	33.7Aa	26.9Ab	19.8ABc	23.7Aa	14.8ABb	15.2Ab
			DM loss			
Control	143.1Bb	104.3Bb	43.4Aa	193.5Ca	129.8Ba	111.1Ba
Lalsil AS	168.4Bb	38.9Aa	24.8Aa	17.9Aa	6.6Aa	8.3Aa
LB 45.22	30.9Aa	33.6Aa	19.9Aa	33.2Aba	2.0Aa	8.4Aa
LB 50.4	96.8ABb	16.1Aa	18.5Aa	127.7BCb	6.0Aa	1.8Aa
LB 90.14	48.6Aa	37.7Aa	16.6Aa	12.2Aa	2.1Aa	5.6Aa

[a-c]Means followed by the same lowercase letter in the row are not different according to the Tukey's test ($P > 0.05$).

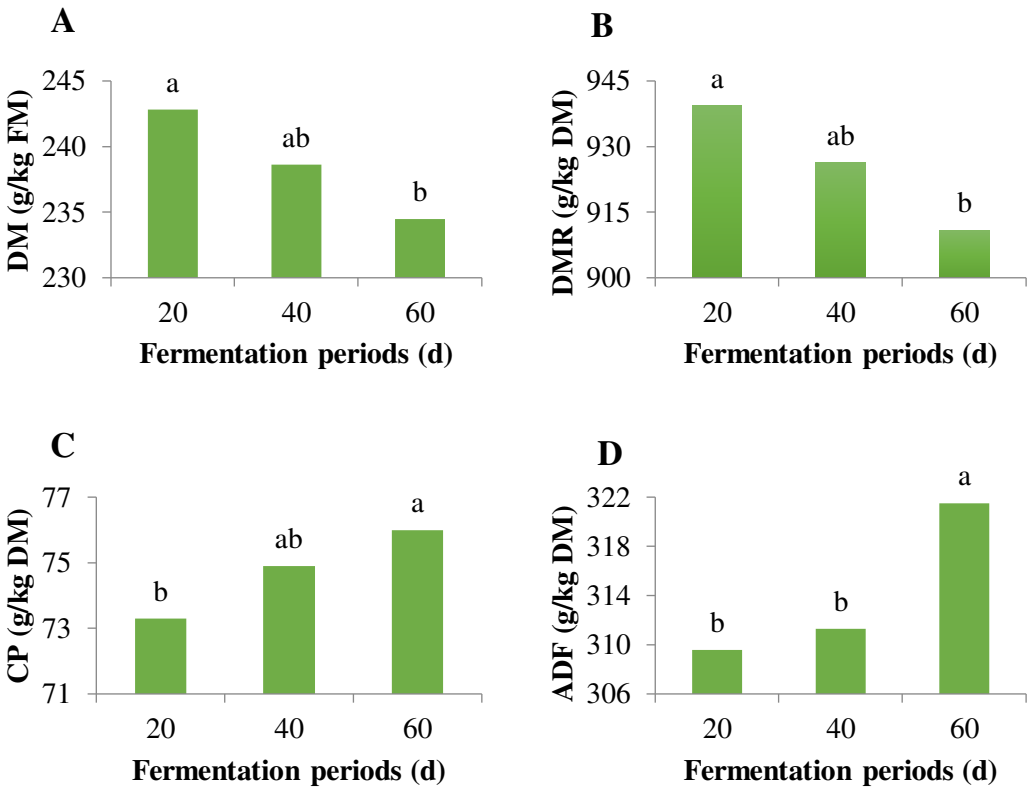
[A-D]Means followed by the same uppercase letter in the columns are not different according to the Tukey's test ($P > 0.05$).

[¶] Control = no inoculant, Lalsil AS = commercial inoculant containing *L. buchneri* CNCM I-4323, LB 45.22 = *L. buchneri*; strain 45.22, LB 50.4 = *L. buchneri*; strain 50.4, LB 90.14 = *L. buchneri*; strain 90.14.

[#] MTR = Maximum temperature reached

[§] ND = not detected

[¥] WSC = water-soluble carbohydrates



Figure

Figure 1. Effect of fermentation periods on dry matter (DM; A), DM recovery (DMR; B), crude protein (CP; C) and acid detergent fiber (ADF; D) of sorghum silage. Means of the two years of evaluation. [a-b]Means followed by the same letter are not different according to the Tukey's test ($P > 0.05$).

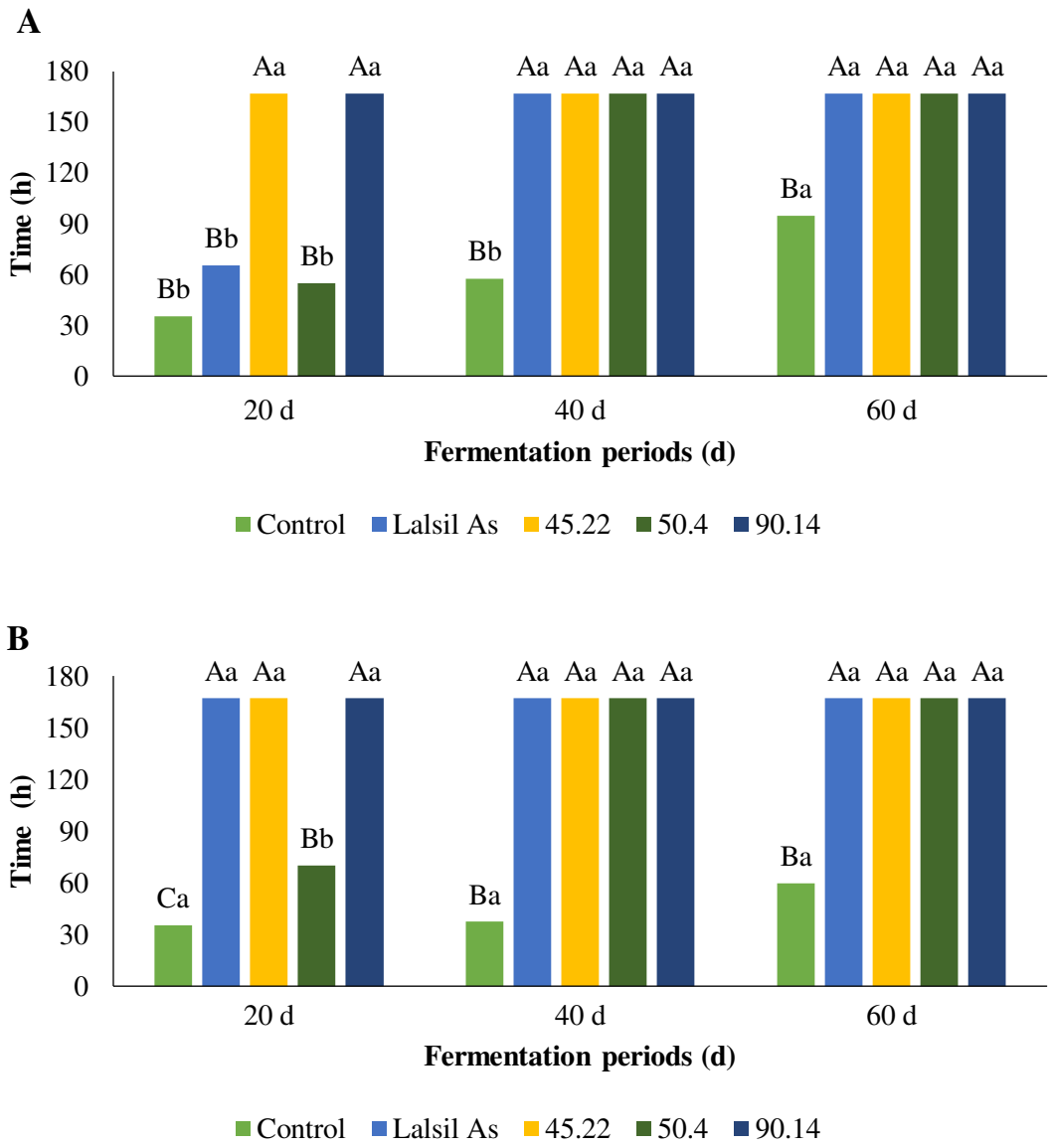


Figure 2. Effect of different strains of *Lactobacillus buchneri* on aerobic stability of sorghum silage ensiled for 20, 40 and 60 d in years 1 (A) and 2 (B). Means followed by the same letter are not different according to the Tukey's test (P > 0.05). Lowercase letters compare the same treatments along different fermentation periods and uppercase letters compare treatments within each fermentation period.

4. CHAPTER II: Effect of new strains of *Lactobacillus buchneri* as inoculant in sorghum silage on the fermentative profile, aerobic stability and voluntary intake in lambs

Abstract: We evaluated the effect of different wild strains of *Lactobacillus buchneri* on the fermentation and aerobic stability of sorghum silages, as well as intake, digestibility, nitrogen balance and ruminal fermentation of lambs fed these silages. Four Santa Inês x Dorper crossbred male, fistulated in the rumen, were distributed in a 4 × 4 Latin square design. Animals were fed the following sorghum silages as a forage source: silage without inoculant (control); silage inoculated with a commercial inoculant (Lalsil As); silage inoculated with the *L. buchneri* strain LB 50.4 (LB 50.4) and silage inoculated with the *L. buchneri* strain LB 90.14 (LB 90.14). The experimental period lasted 20 d. All inoculated silages and their TMR remained stable over 7 d of air exposure, while the control silage and its TMR lost their aerobic stability after ±40.96 h ($P < 0.001$). There was no effect of inoculant on nutrient intake ($P \geq 0.106$). The diet containing LB 50.4 inoculated silage showed the lowest digestibility of dry matter ($P = 0.037$), organic matter ($P = 0.037$) and non-fibrous carbohydrates ($P = 0.012$). The lowest digestibility of dry matter ($P = 0.037$), organic matter ($P = 0.037$) and non-fiber carbohydrates ($P = 0.012$) were observed for the diet containing LB 50.4 inoculated silage. Crude protein digestibility was higher for the diet containing LB 90.14 inoculated silage ($P = 0.036$). The LB 50.4 silage-based diet showed the highest acetate: propionate ratio ($P = 0.005$). Strains of *L. buchneri* used in the present study were efficient in improving the aerobic stability of sorghum silage and their respective diets, without compromising nutrient intake, as well as ruminal fermentation of lambs fed these diets, showing great potential to be used as inoculants in sorghum silages under tropical conditions.

Keywords: acetic acid, aerobic stability, digestibility, wild strains

4.1. Introduction

Globally, silage is the main source of roughage used in the diet of ruminants, and different additives are used to improve fermentation, reduce losses, and increase the aerobic stability (**AS**) of silage (Muck et al., 2018). Among these additives, *Lactobacillus buchneri*, an obligate heterofermentative lactic acid bacterium (**LAB**)

(Heinl et al., 2012; Oude Elferink et al., 2001), has been most commonly studied. It produces acetic acid during the degradation of lactic acid, reducing the population of yeasts, the main microorganisms involved in the initiation of aerobic deterioration (Danner et al., 2003; Driehuis et al., 1999).

Several studies involving the isolation of new strains of LAB have been carried out under tropical conditions (Agarussi et al., 2022; Ávila et al., 2009; Silva et al., 2018), mostly focusing on the evaluation of the fermentative profile, the chemical composition, and the AS of the silage. However, when selecting new strains, it is necessary to evaluate the effects of inoculated silages on animal feed as inoculation can change the odor of the silage (Zhang et al., 2021), therefore potentially altering its acceptance by the animals (Scherer et al., 2019).

The increase in acetic acid concentrations in the silage is the main effect observed in silages inoculated with *L. buchneri* (Arriola et al., 2021). Although acetic acid has been reported to inhibit consumption, relevant data are controversial, as observed in two recent meta-analyses (Arriola et al., 2021; Gerlach et al., 2021). Gerlach et al. (2021) observed that in dairy cattle, the increase in acetic acid in the total mixed ration (**TMR**) reduced the consumption of dry matter (**DM**). On the other hand, Arriola et al. (2021), also in a meta-analysis, evaluated the effects of inoculation of *L. buchneri* in silages on the performance of dairy cows and found no impact of inoculation on DM intake or milk production.

In this study, we tested the hypothesis that strains of *L. buchneri* alter fermentation and improve the AS of sorghum silage, as well as its TMR, which may result in different responses of nutrient intake and digestibility. The objective of this study was to evaluate the fermentative profile, chemical composition, and AS of sorghum silage inoculated with new wild strains of *L. buchneri*, as well as intake, apparent digestibility, nitrogen balance, and ruminal fermentation in lambs fed a diet based on these silages.

4.2. Material and methods

The experiment was conducted in the Animal Science Department of the Federal University of Viçosa, Viçosa, Brazil, MG. The protocol number approved by the Ethics Committee for Animal Use of the Federal University of Viçosa was CEUAP/DZO/UFV 041/2019.

2.1. Planting, harvesting, and ensiling

Forage sorghum (*Sorghum bicolor* (L.) Moench cv. Podium, Biomatrix®, Minas Gerais, Brazil) was cultivated in an area of 2,000 m^2, with a line spacing of 0.8 m. At the time of sowing, 202 kg/ha of an N-P-K mixture (8-28-16) was applied, and after 45 d, topdressing was carried out with 70 kg/ha of N, using urea. The sorghum was harvested manually, 117 d after planting, when the grain was in the floury stage, and the material was ground in a stationary forage machine (PN Plus 2000 model, Nogueira S.A., São João da Boa Vista, São Paulo, Brazil) to an average theoretical particle size of 1.5 cm. The ground material was divided into four piles of 550 kg each (one pile per treatment), with the following treatments: silage without inoculant (**control**); silage inoculated with commercial inoculant (**Lalsil As**); silage inoculated with *L. buchneri* strain LB 50.4 (**LB 50.4**); and silage inoculated with *L. buchneri*, strain LB 90.14 (**LB 90.14**). All inoculants were diluted in 250 mL of distilled water and applied with the aid of hand sprayers at a dose of 1×10^6 CFU/g of fresh matter (**FM**). The same volume of distilled water was applied to the control silage.

The commercial inoculant used was Lalsil As (Lallamend, Goiás, Brazil), which contains the strain *L. buchneri* CNCM I-4323. The wild strains LB 50.4 and LB 90.14 belong to the collection of microorganisms of the Laboratory of Silage Microbiology, Department of Animal Science-UFV, previously isolated in sorghum silages after 56 and 90 d of fermentation, respectively. The sequences of the strains are deposited in the GenBank database under the following access numbers: *L. buchneri* 50.4 - MH924298 and *L. buchneri* 90.14 - MH936314.1.

Prior to the day of ensiling, wild strains of *L. buchneri* stored at -80°C were cultivated in 3 mL of MRS broth (Merck, Darmstadt, Germany) at 37°C for 16 h and subsequently re-cultivated in 5 mL of MRS broth at 37ºC for 16 h. After the second cultivation, serial dilutions were performed, followed by plating on MRS agar medium (DifcoTM Lactobacilli MRS Agar®, São Paulo, Brazil). The plates were incubated for 48 h in BOD at 37°C to determine the number of colony-forming units (**CFU**)/mL of MRS broth. Based on this result, the final volume of MRS broth in which the strains should be cultivated to reach the application rate of 1×10^6 CFU/g of sorghum was determined. Once the required volume of MRS broth was determined, the strains were cultured again for 16 h in BOD at 37°C, and at the end, the culture tubes were centrifuged at 1,000 × g × 10 min, and the supernatant was discarded. The pellets

containing the cells were resuspended in 250 mL of distilled water for application to the crushed material.

The treated material was ensiled in bags (Silofort, 50 × 110 cm, 200 microns) with ± 23 kg each, with the aid of a packaging machine (Sertaneja Elétrica Gold, Governador Valadares, Minas Gerais, Brazil), sealed, and stored at room temperature, with 24 bags per treatment.

2.2. Fermentation profile and microbial population of silage

During each experimental period of the *in-vivo* digestibility assay, an aqueous extract was obtained from the homogenization of 25 g of silage and 225 mL of sterile solution (Ring Solution®, Oxoid, England) for 1 min in an industrial blender. The aqueous extract was filtered through a double layer of sterile gauze, and the pH was then measured using a digital potentiometer (Tecnal, São Paulo, Brazil). A 15-mL aliquot of the filtered aqueous extract was placed in tubes containing 100 µL of sulfuric acid (50% v/v) and stored at -20ºC.

An aliquot of the aqueous extract was subjected to serial dilutions ranging from 10^{-1} to 10^{-7} to quantify the microbial population. The LAB population was quantified on MRS agar (DifcoTM Lactobacilli MRS Agar®, São Paulo, Brazil), incubated at 37ºC for 48 h. The culture of enterobacteria was carried out on VRB agar (Violet Red Bile Agar®, Oxoid, Basingstoke, UK), incubated at 37°C for 24 h. Yeasts and molds were cultivated in PDA (Potato Dextrose Agar®, Difco, São Paulo, Brazil) acidified with 1.5% (v/v) tartaric acid (10% v/v), at 25°C for 72 and 120 h, respectively. Plates containing between 25 and 300 CFU were considered eligible for counting.

2.3. Aerobic stability of silage and total mixed diet

At the end of the second experimental period of the test with animals, four bags of silage per treatment were randomly selected to carry out the aerobic stability test (206 days of fermentation period). The bags were opened and homogenized, and 2 kg of silage from each bag were placed in 10-L plastic buckets. The aerobic stability of the TMR supplied to the animals was also evaluated, starting from 2 kg of silage, previously mixed with the concentrate, according to the forage: concentrate ratio used in the apparent digestibility assay.

The buckets were stored in a temperature-controlled room (±22°C), and a data logger (iMINI temperature, Cryopak°) was inserted in the geometric center of the silage mass and TMR to measure the temperature every 10 min for 7 d. Two data loggers were distributed in the room to measure the ambient temperature. The AS was defined as the number of hours that the silage or TMR remained stable before the temperature increased to 2°C above room temperature (Kung et al., 2021). At the end of the aerobic exposure period, the buckets were weighed, and the material was homogenized to quantify the mold and yeast population and measure the pH, as described above.

2.4. Animal management and experimental diets

Four castrated Santa Inês x Dorper sheep, fistulated in the rumen (initial weight of 42 ± 3.60 kg) and distributed in a 4 × 4 Latin square were used. The animals were housed in a covered shed, in individual metal metabolic cages, with dimensions of 1.55 x 0.88 x 1.60 m (L x W x H), suspended (0.60 m) and slatted floor, equipped with individual feeders and drinkers and a urine collection system. The animals had previously been dewormed (Albendazole®) and were weighed at the beginning and end of each experimental period, which lasted 20 d, divided into 14 d of adaptation (Machado et al., 2016) and 6 d of sample collection.

The diets had a forage: concentrate ratio of 70:30, based on dry matter (DM), formulated with 15% crude protein (NRC, 2007; Table 1). The animals had free access to clean and fresh water all the time and were fed TMR twice daily (08:00 and 16:00 h), *ad libitum*, allowing for 15% orts (FM basis).

2.5. Intake and digestibility trial

Supplied feed, orts, urine, and feces were collected from the 15th to the 19th day of each experimental period. Total urine collection was performed every 24 h, using plastic buckets containing 100 mL of sulfuric acid (20% v/v), covered with nylon mesh, which were attached to the urine collector in the cages. Urinary volume was measured daily in the morning, and 1% of the urinary volume was sampled, generating a composite sample for each animal in each period. Urine samples were stored in a freezer at -20°C.

The total collection of feces was performed using a nappa bag placed on the animals. The bags were emptied twice daily (7:00 and 15:00 h) and the feces stored

in sealed plastic buckets. Feces were weighed daily in the morning, homogenized, and sampled (10% of the total mass excreted). Likewise, the orts were weighed daily in the morning, homogenized, and sampled in individual plastic bags. Samples of silages and concentrate ingredients were also stored in plastic bags and kept in a freezer (-20ºC). Samples of feces, orts, and feed provided were pre-dried in a forced ventilation oven at 55°C for 72 h and then processed in a knife mill (Tecnal, Piracicaba, São Paulo, Brazil) in a 1-mm sieve. The samples of feces and orts from the 5 d of collection were used to obtain a composite sample, proportional to each collection day based on DM, per animal in each period.

2.6. Ruminal fermentation

Ruminal fluid samples were collected on the last day of each experimental period, before the morning feeding (time 0) and after 3, 6, 9, and 12 h. Ruminal fluid was collected at different points in the rumen-reticulum and filtered through a double layer of gauze. The pH was measured immediately after filtration, using a digital potentiometer (Tecnal, São Paulo, Brazil). For the quantification of ruminal NH_3-N, an aliquot of 40 mL of rumen fluid packed in a plastic bottle containing 1 mL of sulfuric acid (50% v/v) was sampled at all collection times, and for the quantification of VFA (acetate, propionate, and butyrate), only samples from 3 h after feeding were used, where an aliquot of 20 mL of rumen fluid containing 5 mL of metaphosphoric acid (250 g/L) was placed in plastic bottles and stored in a freezer at -20°C.

2.7. Laboratory analyses

Samples of silage, ground corn, soybean meal, orts, and feces, ground to 1 mm, were analyzed for their contents of DM (method 934.01), crude protein (**CP**; method 984.13), ether extract (**EE**; method 920.39), ash (method 942.05), and acid detergent fiber (**FDA**; method 973.18), as described by the AOAC (1990). The organic matter (**OM**) content was obtained by the difference (OM = 100 - ash). Neutral detergent fiber (**NDF**) concentrations were determined with the addition of thermostable α-amylase, without the use of sodium sulfite, according to Van Soest et al. (1991) and modified by Senger et al. (2008) for the autoclave. Subsequently, the NDF residues were corrected for ash contamination (Mertens et al., 2002) and nitrogen compounds (Licitra et al., 1996).

Non-fibrous carbohydrates (**NFC**) were quantified according to Detmann and Valadares Filho (2010), where NFC = OM - CP - apNDF - EE; apNDF is the NDF corrected for ash and residual N compounds. Urine samples were analyzed for the quantification of the total nitrogen content (method 984.13) (AOAC, 1990).

The nitrogen balance was determined as the difference between total nitrogen intake and total nitrogen excreted in the feces (**fecal N**) and in the urine (**urinary N**). The indigestible neutral detergent insoluble fiber (**iNDF**) was measured in samples of silages ground to 2 mm, incubated *in situ* for 288 h in fistulated beef cattle (Valente et al., 2011). After removal, the bags were washed and dried at 55°C for 48 h, and the NDF analysis of the residues was performed as described above.

The aqueous extract of acidified silage was used to evaluate the concentrations of water-soluble carbohydrates (**WSC**; Nelson, 1944) and fermentation products (lactic and acetic acid, and ethanol). For the quantification of silage fermentation products, the samples were treated with calcium hydroxide and cupric sulfate and analyzed on a HPLC according to Siegfried et al. (1984). The HPLC apparatus (SPD-10 AVP, Shimadzu) was equipped with a refractive index detector, using an Aminex HPX-87H column (BIO-RAD, CA, USA), with the mobile phase containing 0.005 M sulfuric acid and at a flow rate of 0.6 mL/min at 50°C.

The concentration of ruminal NH_3-N was obtained as described elsewhere (Okuda et al., 1965), and the products of ruminal fermentation (acetate, propionate and butyrate) were quantified in a Waters Alliance e2695 equipment with a PAD 2998 Detector (photodiode array detector), with system separation consisting of a reversed-phase column C18 ODS 80A (150 x 4.6 mm x 5 µm) with an isocratic mobile phase consisting of 100% aqueous phosphoric acid solution (pH 2.35–2.55); oven temperature was 50°C, with a sample injection volume of 10 µL, a run time of 20 min, and a detector with wavelength excitation at 210 nm.

2. 8. Statistical analysis

Data on chemical composition, fermentative profile, and microbial population (log CFU/g) of silages were analyzed using the SAS 9.4 GLM procedure (SAS System Inc., Cary, NC, USA), according to a completely randomized design, with four repetitions, using the following model:

$$Yij = \mu + Ii + eij,$$

where Yij = dependent variable; μ = overall mean; Ii = inoculant effect; eij = random error, assuming an independent normal distribution (NID), (0; σ2ε).

Aerobic stability data were analyzed using the SAS 9.4 GLM procedure (SAS System Inc., Cary, NC, USA), according to a completely randomized design, in a 2 × 4 factorial scheme, using the following model:

Yijk = μ + Ii + Cj + (I × C)ij + eijk,

where Yijk = dependent variable; μ = overall mean; Ii = inoculant effect; Cj = effect of concentrate addition; (I × C)ij = interaction between factors; eijk = random error, assuming an independent normal distribution (NID), (0; σ2ε).

Data on nutrient intake, apparent digestibility, nitrogen balance, and rumen volatile fatty acids were analyzed using the PROC MIXED procedure of SAS 9.3 (SAS System Inc., Cary, NC, USA), according to a square design, Latin 4 × 4, through the following model:

Yijk = μ + Ii + Aj + Pk + εijk,

where Yijk = dependent variable; μ = overall mean; Ii = fixed effect of inoculant i; Aj = random effect of animal j; Pk = random effect of period k; eijk = random error, assuming an independent normal distribution (NID), (0; σ2ε).

For the variables ruminal pH and ruminal NH_3-N, the analyses were performed using repeated measures over time. The collection times (0, 3, 6, 9, and 12 h after feeding) were repeated in each experimental unit (animal × period). Inoculant (Inoc), collection time (Time), and Inoc × Time interaction were considered fixed effects, whereas animal and period were considered random effects. In case of a significant effect of the Inoc × Time interaction, the unfolding of the inoculant effect within each collection time was performed.

The homogeneity of variances among treatments was assumed, and the variables were subjected to analysis of variance and the Tukey test to compare the means, considering 0.05 as the critical level for the occurrence of type I error.

4.3. Results

3.1. Chemical composition and fermentative profile of silage

There was an effect of the inoculant on the DM, NDFap, ADF, WSC, NFC, and iNDF contents of the silages (Table 2). The DM content ($P < 0.001$) was higher for control silage, whereas the NDFap ($P = 0.003$) and ADF ($P = 0.042$) were higher for silage inoculated with LB 50.4. The silages inoculated with LB 50.4 and LB 90.14

showed the lowest contents of WSC ($P < 0.001$). The NFC content ($P = 0.002$) was lower in silage inoculated with LB 50.4, and the iNDF content ($P = 0.002$) was higher in silage inoculated with LB 50.4 (Table 2).

Inoculation with the LB 50.4 strain resulted in silage with a higher pH ($P < 0.001$) and a lower lactic acid concentration ($P < 0.001$). Acetic acid concentration ($P < 0.001$) was higher in silages inoculated with LB 50.4 and LB 90.14. Silage inoculated with LB 90.14 had the lowest concentration of ethanol ($P = 0.004$) (Table 2).

All inoculated silages showed lower populations of yeast ($P < 0.001$) and molds ($P < 0.001$) in relation to the control silage (Table 2). The BAL population ($P = 0.001$) was higher in silage inoculated with LB 50.4.

3.2. Aerobic stability of silages and TMR

There was an inoculant effect on aerobic stability ($P < 0.001$). All inoculated silages and their respective TMR levels remained stable over 7 d of aerobic exposure (> 168 h), with the lowest maximum temperatures reached ($P < 0.001$). Control silage and its TMR lost aerobic stability with ±40.96 h (Table 3).

There was an effect of the Inoc × Conc interaction on pH ($P < 0.001$). The highest pH values were observed for the TMRs in relation to their silages, except for the TMR based on the LB 50.4 silage, which presented a pH similar to that of its silage. All inoculated silages and their TMRs had a lower pH value than the control silage and its TMR (Table 3).

Yeast and mold counts were only affected by the inoculant ($P < 0.001$). The inoculated silages and their TMRs showed lower yeast and mold counts at the end of 7 d of exposure to air.

3.3. Intake, apparent digestibility, and nitrogen balance

Sorghum silage inoculation did neither affect the DM intake of the diets. expressed in kg/day ($P = 0.338$) or in % BW ($P = 0.214$), nor the nutrient intake of the diets ($P \geq 0.106$; Table 4). However, there was an effect of inoculation on the apparent digestibility of DM, OM, CP, and NFC (Table 5). A higher digestibility of DM ($P = 0.017$) and OM ($P = 0.015$) was observed for diets based on control silage and inoculated with LB 90.14. The diet based on silage inoculated with LB 90.14 showed

a higher (P = 0.036) digestibility of CP, and that based on silage inoculated with LB 50.4 showed a lower digestibility of NFC (P = 0.006; Table 5).

Silage inoculated whit *L. buchneri* did not affect N intake and fecal and urinary N excretion ($P \geq 0.169$; Table 6). However, N retained (P = 0.010), % N retained/N ingested (P = 0.002), and % N retained/N absorbed (P = 0.001) were lower for the silage-based diet inoculated with LB 50.4 (Table 6).

3.4. Rumen fermentation

There was no effect of Inoc × Time interaction on rumen pH (P = 0.233) (Table 7). However, there was an effect of collection time ($P < 0.001$) on this variable. Ruminal pH was reduced after 3 h of feeding in all treatments (Fig. 1).

There was a trend effect for Inoc × Time interaction on ruminal NH_3-N concentrations (P = 0.087; Table 7). The control and silages inoculated with Lalsil As and LB 90.14 showed the highest concentrations of ruminal NH_3-N at 3 h after feeding, whereas for silage inoculated with LB 50.4, the peak of ruminal NH_3-N occurred at 9 h after feeding (Fig. 2).

There was no effect (P = 0.133) of the inoculant on total VFA concentrations (mmol/dL) in the rumen (Table 7). However, silage-based diets inoculated with LB 50.4 had higher concentrations of acetate (P = 0.005) and lower concentrations of propionate (P = 0.003); consequently, this diet resulted in a higher A: P ratio (P = 0.005; Table 7). There was no effect (P = 0.150) of the inoculant on butyrate concentrations.

4.4. Discussion

The inoculation of sorghum with *L. buchneri* altered the chemical composition, the fermentative profile, and the microbial population of the silage. The bacterium *L. buchneri* is an obligate heterofermentative LAB that degrades lactic acid, forming acetic acid, 1,2-propanediol, ethanol, and CO_2 (Heinl et al., 2012; Oude Elferink et al., 2001). However, the rate of lactic acid degradation varies among different strains of *L. buchneri* (Daughtry et al., 2018). The LB 50.4 strain showed a pronounced capacity of lactic acid degradation in relation to the other strains, reflecting a lower concentration of lactic acid in the silage and a higher concentration of acetic acid. This explains the higher pH value of their silage, although it remained

within the range considered adequate for the conservation of the silage mass (Kung et al., 2018).

The higher NDFap and ADF content for silage inoculated with the LB 50.4 strain may be related to the reduction in the DM content of the silage (Carvalho et al., 2014), indicating an intense fermentation of the substrate present, resulting in a higher consumption of WSC and NFC, thereby concentrating the fibrous fraction of the silage. However, even with the highest levels of NDFap, the silage inoculated with LB 50.4 showed an iNDF content similar to that of silage inoculated with Lalsil As.

As expected, inoculation with *L. buchneri* reduced the populations of yeasts and molds in relation to the control silage. This was due to the increase in the levels of acetic acid, which has an antifungal action (Moon, 1983), reducing the survival of yeasts during the storage phase and inhibiting their growth when the silage is exposed to air (Driehuis et al., 1999; Kung et al., 2021).

Yeasts are the main microorganisms involved in aerobic deterioration as some species are tolerant to acidic environments and, when exposed to air, can use lactate as a substrate for their growth (Pahlow et al., 2003). With the reduction of the yeast population, the inoculated silages and their TMR remained stable throughout the 7 d of aerobic exposure. However, the control silage and its TMR lost aerobic stability with ±40.96 h of exposure to air, which presented higher pH values, caused by the consumption of lactic acid by yeasts (Drouin et al., 2021; Jiang et al., 2020; Wilkinson and Davies, 2013) and the highest temperature peaks (±37.90ºC) at the end of 7 d of exposure to air. In this way, the use of an aerobically stable silage would result in a TMR also stable in the trough, allowing the supply of the diet only once a day. This management could improve the farm's operating system without affecting production due to nutrient loss by spoilage microorganisms (Dias et al., 2021; Gheller et al., 2020).

Some authors report that inoculation with *L. buchneri* increases the acetic acid content of silage, which can reduce the DM intake (Anil et al., 1993; Forbes et al., 1992; Gerlach et al., 2021). However, this reduction in voluntary intake could be related to the quality of fermentation, as poorly fermented silages have high concentrations of acetic acid, as well as butyric acid and ammonia, which would exert a greater influence on consumption (Driehuis et al., 2001; Keles and Demirci, 2011). The silages used in our study were all well fermented, within the standard range for

sorghum silages (Filya, 2003; Rodrigues et al., 2020), which may explain the lack of an effect of inoculants on nutrient consumption. However, in a recent meta-analysis, Gerlach et al. (2021) observed that in dairy cattle, in increase in acetic acid by 1 g per kg of DM from TMR reduced DM intake by 1.2 g (per 100 kg BW). When the acetic acid content exceeded 17.3 g/kg DM in the TMR, the reduction in consumption was 5.6 g for every 1 g more acetic acid in the TMR (Gerlach et al., 2021). This meta-analysis used data from experiments in which acetic acid was supplied to the animals in its pure form and not only from fermentation by *L. buchneri*, thus reaching higher levels of acetic acid in the diet (60 g/kg of DM). On the other hand, Arriola et al. (2021), also in a meta-analysis, evaluated the effect of inoculation of *L. buchneri* on the performance of dairy cows and did not observe any effect of inoculation on DM intake and milk production. According to Rabelo et al. (2018a), the consumption of diets prepared with inoculated silages depends on several factors since different species and strains, as well as the changes caused in silage fermentation and in the rumen, can result in different impacts on consumption.

The higher digestibility of DM and OM for the control silage and inoculated with LB 90.14 is possibly due to the lower concentration of iNDF of these silages in relation to LB 50.4, which presented the lowest digestibility of these fractions. Corroborating our data, Hristov et al. (2020) observed a negative correlation between OM digestibility and the iNDF content of the diet.

In a meta-analysis, Schuba et al. (2017) observed a positive correlation between the increase in N intake and nitrogen excretion in feces and urine in different ruminant species. This may explain the lack of an effect of diets on N intake and N excretion in feces and urine in our study as the diets were formulated to be isoprotein, and nutrient intake was similar among diets. However, the amount of N retained in animals fed a diet containing silage inoculated with the LB 50.4 strain was lower than that for the other diets. Nkosi et al. (2015, 2010) observed a higher N retention in lambs fed with inoculated silages compared to the control, attributing this effect to the better digestibility of CP. In part, this response was also observed in our study, with higher N retention for animals fed diets that showed higher CP digestibility. However, the use of inoculants (except LB 50.4, which reduced N retention) did not change the amount of N retained in relation to control silage.

Regarding ruminal NH_3-N, all diets showed values above 8 mg/dL, the minimum value recommended by Detmann et al. (2009) to meet the physiological

needs of microorganisms in the rumen under tropical conditions. On the other hand, the non-alteration of the ruminal pH of the animals in the different diets can probably be explained by the similar concentration of VFA in the rumen (Rabelo et al., 2018b; Basso et al., 2018), although this was affected by time, reducing at 3 h after feeding, depending on the intake of easily digestible nutrients.

The higher A: P ratio in animals receiving a silage-based diet inoculated with LB 50.4 resulted from the change in the molar proportion of these acids in the rumen, with an increase in the proportion of acetate and a reduction of propionate for this diet when compared to the others. The higher fibrous fraction and lower NFC digestibility of this diet resulted in lower rumen propionate formation (Rabelo et al., 2018b; Wang et al., 2020) or even due to the lower lactic acid concentration of this silage compared to the others, as in the rumen, lactic acid is quickly converted to propionate (Charmley, 2001; Cherdthong et al., 2021).

4.5. Conclusions

The strains of *L. buchneri* used in the present study could efficiently improve the aerobic stability of sorghum silages and their respective TMR, without compromising nutrient intake, as well as rumen fermentation in lambs. Therefore, it can be inferred that the wild strains LB 50.4 and LB 90.14 have great potential to be used as inoculants in sorghum silages under tropical conditions.

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Figure 1. Effect of inoculation of sorghum silage with *L. buchneri* on ruminal pH at different intervals post-feeding. Control = silage without inoculant; Lalsil As = silage inoculated with commercial inoculant Lalsil As; LB 50.4 = silage inoculated with the strain LB 50.4; and LB 90.14 = silage inoculated with the strain LB 90.14.

Figure 2. Effect of sorghum silage inoculation with *L. buchneri* on ruminal NH_3-N at different intervals post-feeding. Control = silage without inoculant; Lalsil As = silage inoculated with commercial inoculant Lalsil As; LB 50.4 = silage inoculated with the strain LB 50.4; and LB 90.14 = silage inoculated with the strain LB 90.14.

Figure 1.

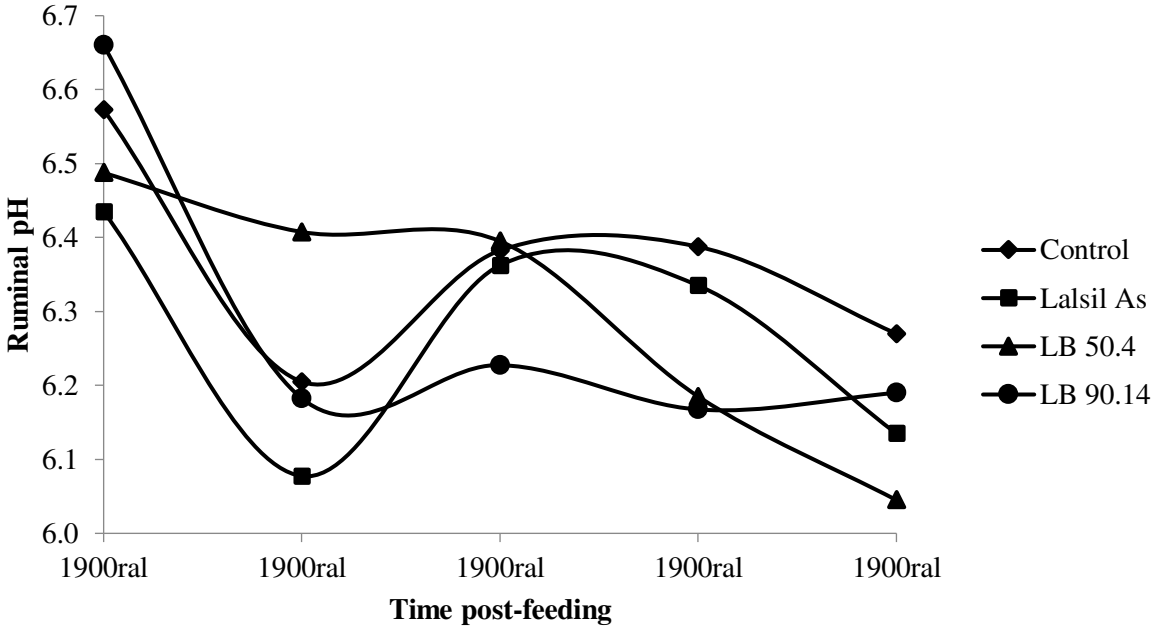

Figure 2.

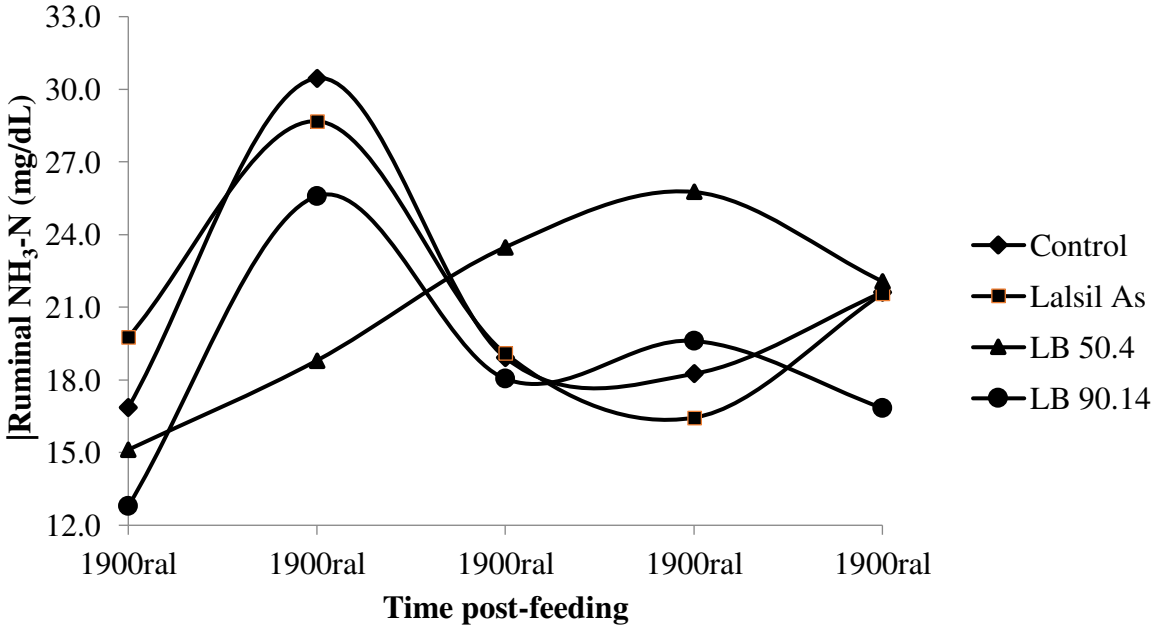

Table 1. Proportion of ingredients and chemical composition of experimental diets.

	Diets[1]			
	Control	Lalsil As	LB 50.4	LB 90.14
Ingredients proportion (g/kg of DM[2])				
Sorghum silage	700	700	700	700
Ground corn	170.2	170.2	170.2	170.2
Soybean meal	104.8	104.8	104.8	104.8
Urea[3]	8.3	8.3	8.3	8.3
Mineral premix[4]	16.7	16.7	16.7	16.7
Chemical composition (g/kg of DM)[5]				
DM (g/kg of FM)	431.6	423.5	412.4	424.1
OM	941.4	942.7	939.1	941.8
NDFap	427.9	417.7	449.8	414.9
CP	154.3	152.3	153.1	150.9
EE	36.9	37.4	34.8	34.9
NFC	352.1	366.6	331.5	372.4

[1]Control- sorghum silage without inoculant; Lalsil As- sorghum silage inoculated with the commercial inoculant Lalsil As; LB 50.4 – sorghum silage inoculated with the strain of *Lactobacillus buchneri* - LB 50.4; LB 90.14 - sorghum silage inoculated with the strain of *Lactobacillus buchneri* – LB 90.14.

[2]DM- dry matter.

[3] Urea + ammonium sulfate in a 9 :1 ratio.

[4] Mineral premix: 278 g/kg of calcitic limestone, 588 g/kg of dicalcium phosphate, 120 g/kg of sodium chloride, 14 g/kg of Premix (content: 167,7 g/kg of copper sulfate, 812 g/kg of zinc sulfate, 7,7 g/kg of cobalt sulfate, 6.2 g/kg of potassium iodate and 6.4 g/kg of sodium selenite).

[5]FM- fresh matter; OM- organic matter; NDFap- neutral detergent fiber corrected for ash and protein; CP- crude protein; EE- ether extract; NFC- non-fiber carbohydrates.

Table 2. Chemical composition, fermentation profile and microbial population of sorghum silages inoculated with different strains of *Lactobacillus buchneri*.

Items[2]	Inoculants[1]				SEM[3]	P-value[4]
	Control	Lalsil As	LB 50.4	LB 90.14		
Chemical composition (g/kg of DM)						
DM (g/kg of FM)	235.8a	224.2b	208.5c	225.2b	2.626	<0.001
OM	930,3	932,2	927,0	930,9	0.689	0.091
CP	85.1	80	82.9	78.1	1.097	0.102
EE	20.6	21.4	17.6	17.8	0.847	0.283
NDFap	554.4ab	539.8b	585.7a	535.8b	6.186	0.003
ADF	31.4ab	29.9b	33.6a	30.8ab	0.492	0.042
iNDF	20.83ab	20.02b	21.76a	19.74b	0.243	0.002
NFC	270.3ab	291.0a	240.8b	299.3a	6.961	0.002
WSC	19.0a	28.4a	6.9b	7.3b	2.513	<0.001
Fermentation profile (g/kg of DM)						
pH	3.64b	3.59b	3.85a	3.62b	0.030	<0,001
Lactic acid	101.6a	104.2a	48.8b	81.6a	0.665	<0.001
Acetic acid	10.6b	27.7b	59.9a	51.5a	0.626	<0.001
Ethanol	12.3ab	13.6a	18.3a	6.8b	0.125	0.004
Microbial population (log CFU/g)						
LAB	7.52ab	6.75bc	7.76a	6.21c	0.186	0.001
Yeast	5.07a	3.04b	3.13b	2.65b	0.261	<0.001
Mold	3.35a	2.26b	2.27b	<2.0b	0.177	<0.001
Enterobacteria	< 2.0	< 2.0	< 2.0	< 2.0	-	-

[1]Control- sorghum silage without inoculant; Lalsil As- sorghum silage inoculated with the commercial inoculant Lalsil As; LB 50.4 – sorghum silage inoculated with the strain of *Lactobacillus buchneri* - LB 50.4; LB 90.14- sorghum silage inoculated with the strain of *Lactobacillus buchneri* – LB 90.14.

[2]DM- dry matter; FM- fresh matter; OM-organic matter; CP- crude protein; EE- ether extract; NDFap- neutral detergent fiber corrected for ash and protein; ADF- acid detergent fiber; iNDF- indigestible neutral detergent fiber; NFC- non-fiber carbohydrates; WSC- water-soluble carbohydrates; LAB- lactic acid bacteria.

[3]Standard error of the mean.

[4]Probability of inoculant effect. Means followed by the same letter on the line do not differ by Tukey's test at 0.05 probability for type I error.

Table 3. Aerobic stability, pH, mold and yeasts of sorghum silage inoculated with different strains of *Lactobacillus buchneri*. or its TMR after 7 days of aerobic exposure.

	Inoculant[1]					P-value[2]		
	Control	Lalsil As	LB 50.4	LB 90.14	SEM[3]	Inoc	Conc	Inoc*Conc
	Aerobic stability (h)				9.885	<0.001	0.214	0.209
Silage	42.46	168	168	168				
TMR	39.46	168	168	168				
Average	40.96b	168a	168a	168a				
	Maximum temperature reached (ºC)				1.202	<0.001	0.766	0.994
Silagem	38.05	22.53	22.225	23.65				
TMR	37.75	22.3	22.3	23.5				
Average	37.90a	22.41b	22.26b	23.56b				
	pH				0.292	<0.001	<0.001	<0.001
Silagem	6.68Ba	3.55Bb	3.79Ab	3.60Bb				
TMR	8.01Aa	3.69Ab	3.92Ab	3.74Ab				
	Yeast (log CFU/g)				0.394	<0.001	0.772	0.579
Silagem	8.77	4.63	4.03	3.8				
TMR	8.78	4.13	4.59	3.36				
Average	8.78a	4.38b	4.31b	3.58b				
	Mold (log CFU/g)				0.368	<0.001	0.195	0.100
Silagem	7.95	4.14	2.67	3.12				
TMR	7.13	3.16	3.51	2.64				
Average	7.54a	3.66b	3.09b	2.88b				

[1]Control- sorghum silage without inoculant; Lalsil As- sorghum silage inoculated with the commercial inoculant Lalsil As; LB 50.4 – sorghum silage inoculated with the strain of *Lactobacillus buchneri* - LB 50.4; LB 90.14- sorghum silage inoculated with the strain of *Lactobacillus buchneri* – LB 90.14.

[2]Probability of inoculant (Inoc), concentrate addition (Conc) and interaction Inoc with Conc (Inoc*Conc) effects.

Means followed by same lowercase letters in the row and uppercase letters in the columns are not different according to Tukey's test (P > 0.05).

Table 4. Nutrient intake of lambs fed diets containing sorghum silages treated with different strains of *Lactobacillus buchneri.*

Items [2]	Inoculant[1]				SEM[3]	P-value[4]
	Control	Lalsil As	LB 50.4	LB 90.14		
	Intake (g/dia)					
DM	1167.54	1121.4	958.67	1036.07	40.933	0.338
OM	1101.21	1060.05	903.37	978.16	38.626	0.331
NDFap	482.69	467.35	433.61	436.15	13.931	0.586
CP	194.27	180.67	152.17	159.87	7.300	0.169
EE	26.46	26.19	17.88	20.32	1.443	0.106
NFC	422.5	410.46	321.69	383.16	18.299	0.260
TDN	760.71	727.06	578.64	685.28	28.202	0.148
	Intake %BW					
DM	2.75	2.55	2.25	2.5	0.085	0.214
NDFap	1.14	1.07	1.02	1.05	0.027	0.457

[1] Control- sorghum silage without inoculant; Lalsil As- sorghum silage inoculated with the commercial inoculant Lalsil As; LB 50.4 – sorghum silage inoculated with the strain of *Lactobacillus buchneri* - LB 50.4; LB 90.14- sorghum silage inoculated with the strain of *Lactobacillus buchneri* – LB 90.14.

[2] DM- dry matter; OM-organic matter; NDFap- neutral detergent fiber corrected for ash and protein; CP- crude protein; EE- ether extract; NFC- non-fiber carbohydrates; TDN- total digestible nutrients.

[3]Standard error of mean.

[4]Probability of inoculant effect. Means followed by the same letter on the line do not differ according to Tukey's test ($P > 0.05$).

Table 5. Apparent digestibility (g/kg) of nutrients in lambs fed diets containing sorghum silages treated with different strains of *Lactobacillus buchneri*.

	Inoculant[1]					
Items[2]	Control	Lalsil As	LB 50.4	LB 90.14	SEM[3]	P-value[4]
DM	619.20a	609.23ab	570.89b	633.81a	7.394	0.017
OM	648.34a	637.31ab	598.42b	659.19a	7.204	0.015
CP	737.25ab	724.89ab	716.05b	748.35a	5.160	0.036
NDFap	500.49	476.05	466.10	501.94	8.567	0.415
EE	742.66	780.91	696.49	737.21	15.523	0.344
NFC	790.13a	796.13a	742.76b	815.03a	7.934	0.006

[1]Control- sorghum silage without inoculant; Lalsil As- sorghum silage inoculated with the commercial inoculant Lalsil As; LB 50.4 – sorghum silage inoculated with the strain of *Lactobacillus buchneri* - LB 50.4; LB 90.14- sorghum silage inoculated with the strain of *Lactobacillus buchneri* – LB 90.14.

[2]DM- dry matter; OM-organic matter; CP- crude protein; NDFap- neutral detergent fiber corrected for ash and protein; EE- ether extract; NFC- non-fibercarbohydrates;.

[3]Standard error of mean

[4]Probability of inoculant effect. Means followed by the same letter on the line do not differ according to Tukey's test (P > 0.05).

Table 6. Nitrogen balance (g/day) in lambs fed diets containing sorghum silage treated with different strains of *Lactobacillus buchneri*.

	Inoculant[1]					
Items	Control	Lalsil As	LB 50.4	LB 90.14	SEM[2]	P-value[3]
N-intake	31.08	28.91	24.35	25.58	1.168	0.169
N-feces	8.15	7.89	6.91	6.44	0.305	0.196
N-absorbed	22.93	21.02	17.44	19.14	0.900	0.160
Uninary N	15.74	14.39	15.48	12.86	0.577	0.210
N-retained	7.19a	6.63a	1.95b	6.28a	0.669	0.010
N-retained/N intake (%)	22.71a	22.78a	7.93b	24.65a	2.013	0.002
N-retained/N-absorbed	30.76a	31.34a	11.13b	32.96a	2.675	0.001

[1]Control- sorghum silage without inoculant; Lalsil As- sorghum silage inoculated with the commercial inoculant Lalsil As; LB 50.4 – sorghum silage inoculated with the strain of *Lactobacillus buchneri* - LB 50.4; LB 90.14- sorghum silage inoculated with the strain of *Lactobacillus buchneri* – LB 90.14.

[2]Standard error of mean.

[3]Probability of inoculant effect. Means followed by the same letter on the line do not differ according to Tukey's test (P > 0.05).

Table 7. Ruminal fermentation of lambs fed diets containing sorghum silages inoculated with different strains of *Lactobacillus buchneri*.

	Inoculant[1]					P-value[4]		
Items[2]	Control	Lalsil As	LB 50.4	LB 90.14	SEM[3]	Inoc	Time	Inoc*Time
pH	6.36	6.27	6.24	6.29	0.028	0.237	<0.001	0.233
NH_3-N	21.22	21.11	21.05	18.58	0.892	0.233	<0.001	0.087
Total	7.8	8.07	5.78	6.89	0.365	0.113		
Molar proportion								
Acetate	76.18b	75.03b	80.96a	76.34b	0.688	0.005		
Propionate	18.58a	19.75a	15.32b	17.69a	0.529	0.003		
Butyrate	5.24	5.23	3.72	5.97	0.344	0.150		
A:P	4.13b	3.81b	5.36a	4.33b	0.190	0.005		

[1]Control- sorghum silage without inoculant; Lalsil As- sorghum silage inoculated with the commercial inoculant Lalsil As; LB 50.4 – sorghum silage inoculated with the strain of *Lactobacillus buchneri* - LB 50.4; LB 90.14- sorghum silage inoculated with the strain of *Lactobacillus buchneri* – LB 90.14.

[2]NH_3-N- ammonia nitrogen, mg/dL; Total VFA- total volatile fatty acids, mmol/dL; Molar proportion- mmol/100 mmol, A:P- acetic:propionic acid ratio.

[3]Standard error of mean.

[4]Probability of inoculant (Inoc), collection time (Time) and interaction Inoc with Time (Inoc*Time) effects. Means followed by the same letter on the line do not differ according to Tukey's test ($P > 0.05$).

5. CONCLUSÕES GERAIS

A inoculação com a cepa LB 45.22 e 90.14 apresentam grande potencial como inoculantes em silagem de sorgo, pois foram eficientes em incrementar a estabilidade aeróbia em um curto tempo de fermentação nos dois anos de estudos. A cepa LB 50.4 e o inoculante comercial também foram eficientes em melhorar a estabilidade aeróbia, entretanto, requerem um maior tempo de fermentação. Ao serem avaliadas seu efeito sobre o consumo animal, as cepas LB 50.4 e 90.14 não reduziram o consumo de MS e nutrientes digestíveis totais, além disso, foram também capazes de melhorar a estabilidade aeróbia da silagem em silos em escala maior.